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A Summary of Current Program, 7/1/62

and Preliminary Report of Progress

for 1/1/61 to 6/30/62

ANIMAL DISEASE AND PARASITE RESEARCH DIVISION

of the

AGRICULTURAL RESEARCH SERVICE

UNITED STATES DEPARTMENT OF AGRICULTURE

This progress report of U.S.D.A. and cooperative research is primarily a tool for use of scientists and administrators in program coordination, development and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

There is included under each problem area in the report a brief and very general statement on the nature of the research being conducted by the State Agricultural Experiment Stations and the professional manpower being devoted by the State stations to such research. Also included is a brief description of related work conducted by private organizations. No details on progress of State station or industry research are included except as such work is cooperative with U.S.D.A.

The summaries of progress on U.S.D.A. and cooperative research include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued between January 1, 1961, and June 30, 1962. Current agricultural research findings are also published in the monthly U.S.D.A. publication, Agricultural Research. This progress report was compiled in the Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland.

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INTRODUCTION

Animal disease and parasite research, as used in this report, is concerned with infectious, non-infectious, and parasitic diseases of cattle, swine, sheep, goats, horses, poultry, and fur-bearing animals. It involves fundamental investigations of causes and effects of diseases as they affect economic farm and ranch production.

Man's needs and wants for animal products, and public health and welfare, are inseparably connected with healthy and economically profitable livestock production, at least in the United States. In some parts of the world, animal diseases exist that jeopardize human existence. Fortunately, many animal diseases do not exist in the United States, however, they pose a continuing threat to livestock production in this country. Disease in animals inevitably affects human welfare, either because people become infected or they are deprived of needed food and other products derived from animals. Diseases in the lower animals do not differ essentially from those in man, and frequently infections are interchangeable among them. More than half of the total farm income in the United States is derived from livestock and livestock products. Exact calculation of the extent of reduction of income by animal diseases is difficult. It has been estimated, however, that as many as 1/10 of the domestic animal population is lost each year through incursions by disease in one form or another. Over-all losses due to disease have been estimated to amount to at least $2 billion annually. Such losses must be reduced to minimums if production of meat, milk, leather, wool, and other essential animal products is to keep up with progressively increasing demands.

Expansion of basic research is needed on host-parasite relationships, the relation of chemistry and enzyme systems of causative agents to their pathogenicity, the nature and resistance to disease and methods of breeding resistant animals. Increased studies should be made of growth requirements of disease-causing agents, methods of destroying such agents, methods of diagnosis and treatment of disease, and the detection of carriers of disease. Research at the present level will not be adequate to provide in the '70s the same standard of living enjoyed today. Since the results of research conducted today will not be available, as a general rule, until at least 10 years after the work is started, increases in research are necessary.

The Animal Disease and Parasite Research Division has 38 scientists at the Beltsville Parasitological Laboratory, the National Animal Disease Laboratory, Ames, Iowa, has 92.5 budgeted professional positions, and the Plum Island Animal Disease Laboratory, New York, has 42.5 budgeted positions, in the current fiscal year. The rest of the Division's total complement of 230 budgeted scientific positions, or 58, is distributed among small groups at 11 smaller, specialized field stations, which are located at Auburn, Alabama;

Fontana, California; Denver, Colorado; Live Oak, Florida; Athens and Tifton, Georgia; Albuquerque and State College, New Mexico; Kerrville, Texas, Logan, Utah; Pullman, Washington; and at foreign missions at Amsterdam, Holland, and Kenya, East Africa.

Federally supported research under 51 contracts or cooperative agreements with State stations is equivalent to 12.8 professional man-years.

Analysis of the over-all research effort in the 15 areas of investigations on animal diseases and parasites reveals that basic studies amount to more than half of the work, in terms of professional man-years, and some applied research is carried on at all laboratories and field stations. Depending primarily on the type of work and the kinds of equipment and animals that are required, the average cost of support per professional man-year, in terms of maintenance and operation of facilities, and personal services, varies from approximately $20,000 on some projects, to as much as $80,000 on other projects. Inadequacies of facilities and supporting services at some locations account for comparatively low cost, whereas special facilities and the type of work at others result in inescapably higher cost. The present average cost, amounting to $45,000 per professional man-year, should be increased during the next five years to an average of at least $50,000 per man-year, in order to provide appropriate support in the specialized field of animal disease and parasite research, and for more economical and efficient utilization of funds with fuller satisfaction of research needs.

With the projected supplementation of facilities at the Beltsville Parasitological Laboratory, the National Animal Disease Laboratory, the Plum Island Animal Disease Laboratory, and carefully selected subsidiary field stations, it is now possible for the first time in many years to foresee and project the funds for a comprehensive program of research that is reasonably commensurate with national needs. The basic aim of research in all fields in the United States is protection and satisfaction of human needs. Man's problems are closely related to disease in animals, and expenditures for research on animals should accordingly be more reasonably commensurate.

AREA NO. 1 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF CATTLE

Problem. Losses from infectious and non-infectious diseases of cattle, other than those due to parasites, are estimated at approximately $600 million annually. These losses materially increase costs of production and conversely decrease profits. In turn, they contribute to the cost of every purchase of meat, milk, and other cattle products to the consumer. Some of these diseases are transmissible to man. Determination and definition of the causes of cattle diseases, explorations for efficient methods of diagnosis, prevention, control, and when feasible, eradication, are the purposes of the research program.

USDA PROGRAM

The Department has a continuing long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of cattle. Research is being conducted on the diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 50.7 professional man-years. This effort is divided among sub-headings as follows:

Brucellosis, 2.3 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the universities of Maryland, Minnesota, and Wisconsin.

Paratuberculosis (Johne's Disease), 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Vibriosis, 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreement with the New York State Veterinary College.

Tuberculosis, 4.6 at the National Animal Disease Laboratory, Ames, Iowa, and through contract with the Michigan State University.

Mucosal-Respiratory Disease-Complex, 1.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Indiana and Iowa Experiment Stations (mucosal) and the Colorado State University (respiratory - rhinotracheitis).

Mastitis, 6.2 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California.

Respiratory Disease (Shipping Fever), 5.0 at the National Animal Disease Laboratory, Ames, Iowa.

Leptospirosis, 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Infertility, 3.0 other than vibriosis and trichomoniasis, at the National Animal Disease Laboratory, Ames, Iowa.

Epizootic Bovine Abortion, 3.4 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the University of California.

Enteric Infections, 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Leukosis, 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Foot Rot, 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Keratitis, 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 73.2 professional man-years divided among subheadings as follows: Brucellosis 5.8, vibriosis 6.4, tuberculosis 0.8, mucosal-respiratory disease complex 6.2, mastitis 10, respiratory disease (shipping fever) 4.7, leptospirosis 7, infertility other than vibriosis and trichomoniasis 5.2, epizootic abortion 0.5, enteric infections 2.5, foot rot 0.8, infectious keratitis 2.1, eurolithiasis 1.3, other diseases (clostridia, pulmonary emphysema, white muscle disease, etc.) 17.8. Colorado, Connecticut, Georgia, Kansas, Maryland, Michigan, Minnesota, Ohio, Virginia, and Wisconsin are conducting studies on brucellosis. Seven southern and three northeastern states are working on vibriosis through two regional research projects (S-30, Diseases of Reproduction and NE-40, Pathology of Breeding Failure). Wisconsin and Michigan are conducting research on improving present tests for greater sensitivity in diagnosis of tuberculosis. Eight north central states are conducting research under NC-34 Mucosal Disease. Florida coordinates related work on an informal basis with the north central states on mucosal-respiratory disease complex. North central, northeastern, southern, and western regions are all conducting research on mastitis. Seven north central states and the Department are cooperating through regional research (NC-34, Shipping Fever of Cattle). Five southern and two northeastern states cooperate in regional research (S-30, Diseases of Reproduction, and NE-40, Pathology of Breeding Failure). Cooperative regional studies among four northeastern states (NE-40, Pathology of Breeding Failure) and four southern states (S-30, Diseases of Reproduction) are being made on infertility, other than vibriosis and trichomoniasis. California, in cooperation with the Department, is conducting research on epizootic abortion. Arizona, Connecticut, Colorado, and Missouri are studying bacteria and viruses found associated with intestinal infections of cattle, known as enteric infections. Colorado is conducting research on foot rot. Arizona, Kansas, Montana, Nebraska, Oklahoma, and Texas are conducting research on infectious keratitis. Five western states are cooperating in regional research (W-41, Urinary Calculi of Beef Cattle)- Urolithiasis. All regions are conducting some research on Other Diseases (clostridia, pulmonary emphysema, white muscle disease, etc.)

Industry and other organizations are engaged in the preparation of marketable biologic and pharmaceutical products. They conduct experimentation on vaccines and the formulation of chemical compounds and other medicinal substances for prevention and treatment of diseases of cattle. These companies generally will utilize their own facilities. Information gained in their research generally is confidential in nature as are expenditures for research and development. It is estimated that 80 professional man-years are devoted to this work by industry and other organizations.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Brucellosis

In 1961, at the Animal Disease Station, Beltsville, Maryland, the persistence and localization of Brucella abortus infection in 169 bovine females was determined. Major sites of localization were the udder and supermammary lymph glands. Infection was shown to persist up to 11 years in these locations.

After exposure to virulent Brucella abortus strain 2308, cattle produced two types of sero-agglutinins. These could be differentiated on their ability to withstand heat (65 C for 15 minutes). Subsequently the relative percentages and the persistence of the two types of seroagglutinins differed between the resistant cattle and the cattle that became infected. In general, resistant cattle produced a small percentage of heat stable sero-agglutinins which were limited to the first 70 days after exposure. However, cattle that became infected produced predominantly heat stable sero-agglutinins throughout the course of the disease.

Acidified plate test antigens were shown to be incapable of differentiating serologic reactions of infected cattle from those of Brucella resistant cattle. The tests were not selective but rather the results depended upon the magnitude of the titer to the standard sero-agglutination test and the pH of the antigen employed.

An experimental complement fixation test was less effective than the heat inactivation test or the standard sero-agglutination tests for the detection of infection in the immediate post-exposure period, except in a few animals that became infected but did not abort.

The potential value of infra-red spectroscopy as means of identifying and classifying typical and atypical strains of the genus Brucella was investigated further.

Five hundred and seventy-five apparently normal fetuses, asceptically obtained, were studied from a bacteriological point of view. Serological tests, direct cultures on several media, as well as inoculation into embryonating eggs and guinea pigs, showed that bacteria were generally present in embryos and that the fetal skin seemed to harbor more microorganisms than other tissues and organs in the fetus.

Brucella isolations were made from the fetuses of negative cows and from some having titers of 1:2000 and greater. Fetuses of every age group harbored Brucella, and organisms were isolated from about 4 per cent of all fetuses examined. More than half of the isolations were made from the skin. No Leptospira were isolated at any time.

Immunizing agents have been studied in an attempt to develop a satisfactory vaccine for adult cattle and over-age calves. Strains of Brucella of reduced infectivity for laboratory animals have been produced using (a) streptomycin-dependent strains, adapted to living on 1000_u per ml. for 1 year and then adapted to growing without the antibiotic, and (b) embryonating egg-adapted strains. The latter have been serially passed through embryonating eggs from 100 to 500 times, and are highly potent in eggs. A very mild disease is produced in laboratory animals, but following challenge they exhibited a high degree of resistance to infection with virulent forms of the organism.

In 1962, at the National Animal Disease Laboratory (NADL), Ames, Iowa, experiments were completed to determine immunogenic response of calves vaccinated at different ages with Brucella abortus Strain 19. Midway through their first gestation 69 vaccinated cattle and 22 nonvaccinated controls were exposed conjunctivally to approximately 7×10^5 cells of virulent Brucella abortus Strain 2308. Proof of infection was based upon isolation of the organism from one or more of the following sources: milk, dam's blood, uterine contents and fetus at parturition, or tissues obtained at necropsy.

Eight of 24 calves vaccinated at 4 months of age became infected and 5 aborted. Eight of 22 calves vaccinated at 6 months of age became infected and 5 aborted. Seven of 23 calves vaccinated at 8 months of age became infected and 6 aborted. Twenty of 22 nonvaccinated controls became infected and 15 aborted.

Although no significant differences in the degree of vaccinal immunity could be shown among the three groups of cattle vaccinated at different ages, vaccination at 4 months of age materially reduced the problem of persistent postvaccinal titers.

Natural infection with Brucella abortus in 2 bulls was studied over a period of several years. Serologic, bacteriologic, and histopathologic examinations were correlated with the clinical manifestations of the disease.

Seroagglutinin and semen plasma agglutinin titers persisted for at least 5 years. Brucella abortus was consistently isolated from the semen of both bulls throughout the course of the disease.

At necropsy Brucella abortus was isolated from the testes, epididymides, seminal vesicles and the ampullae of the ductus deferens.

Pathologic changes occurred throughout the genital tract. Granulomas, including sperm granulomas, were found in the epididymis of one bull.

The high percentage of ejaculates containing viable Brucella abortus emphasized the need for frequent bacteriologic examinations of semen for evidence of Brucella abortus infection in all bulls used for artificial insemination or natural service.

A modified Coombs' test for the detection of incomplete antibodies was evaluated on serum frozen and stored at dry ice temperature from a previous exposure of vaccinated and nonvaccinated cattle. The test failed to reveal the presence of any incomplete antibodies during the course of the disease.

The conglutinin-complement absorption test was also evaluated on the same lots of frozen and stored serum. Under the conditions and limitations of the experiment the conglutinin-complement absorption test appeared to have no advantage over the standard seroagglutination tube and plate tests or supplementary tests (heat inactivation, complement-fixation, or acidified plate antigens). Furthermore, the complexity of the test rendered it less adaptable for a routine diagnostic laboratory tool.

The entire stock culture collection was repacked and recataloged for the move to the new Ames laboratory, consequently all lyophilized cultures are more readily accessible for future research purposes.

A critical study of the tube agglutination test for the diagnosis of brucellosis in cattle has been inaugurated within the last few months. Sufficient data has not been obtained on any of the variable factors to submit results at this time, but considerable information has been accumulated. Perhaps a better testing procedure may not result from this investigation, but it is possible that some minor changes could be made, which might improve the test.

An attempt has been made to develop a vaccine satisfactory for adult animals and over-age calves, which will not produce a lasting titer. Streptomycin-adapted strains, grown with 20,000 micrograms per milliliter of the antibiotic over a period of time, have been used for guinea pig vaccination. These strains are not streptomycin-dependent, but have been modified in growing with the antibiotic to such an extent that vaccinal titers produced by them are low and transient.

Six embryonating egg-adapted strains of Brucella are being serially carried forward in eggs, one strain having been passed 626 times and others through a lesser number of transfers. Results of trials with egg-adapted Strain 19 (175th passage) and B.C. (600th passage) indicate that both strains have some value as immunizing agents.

Experimental work on the protective quality of cattle blood serum has been confined to further study of 106 vaccinated heifers, which have been tested at monthly intervals.

In 1961, at the University of Maryland under a cooperative agreement with the USDA, studies on Actomer were continued regardless of the toxic effect of the acetone in which it must be dissolved. Experiments, using Actomer in guinea pigs have shown the drug to be effective for Brucella, even in very dilute solutions.

In 1962, Actomer was further studied in relation to its solubility in non-toxic agents. Mice have been used for the in vivo experiments.

In 1961, at the University of Minnesota under a cooperative agreement with the USDA, the experimental work on the protective qualities of cattle blood serum against Brucella was confined to a study of the serum obtained from calves and heifers. Data has been accumulated to complete the results on more than 2000 animal sera.

A Brucella agglutinating component (11.7 S) has been isolated from the milk of 2 cows and appears to differ in several major respects from the brucella agglutinating macroglobulin (19 S) of blood serum.

Epidemiologic, serologic, and bacteriologic studies of Brucellosis "Problem Herds" have been of material assistance in establishing 7 brucellosis-free counties in Minnesota during the past 8 months.

A differential diagnostic test was developed which may be useful in differentiating "non-specific" and "specific" agglutinins for brucella in blood serum and this new test is now undergoing further evaluation.

In 200 herds with long histories of brucella infection, the plate sero-agglutination test failed to identify almost 60 percent of probably-infected cows. In 681 herds, without such a long-standing history of infection, the plate test failed to identify only 16 percent of probably-infected animals. In the problem herds, the combination of plate, tube and whey agglutination tests properly identified 40 of 46 animals proved bacteriologically to be infected.

Herds with consistently positive ring tests which fail to disclose reactors to the plate sero-agglutination test have also been investigated. In most cases, a single animal has been found to be causing the ring test reaction. All were identified by the combination of plate, tube and whey tests.

In 1962, at the University of Minnesota under a cooperative agreement, studies of nonspecific agglutinins (NSBA) in bovine milk were continued to develop improved methods of isolation and increase the yield for further characterization of the macroglobulins. Studies on the use of mercaptoethanol to detect macroglobulins (NSBA) in bovine milk were inaugurated using the procedures which were developed last year.

Studies on mercaptoethanol to detect macroglobulins (NSBA) in blood serum of cattle, which react to the tube sero-agglutination test at dilutions of 1:25 or greater, are being continued in the development of a new procedure which may be useful in the field.

A Modified Cream Ring Test has been developed as a result of studies during 1960-61 which showed that the present cream ring test may fail to detect brucella agglutinins in up to 50% of cream samples tested when such cream has partially deteriorated or soured prior to sampling. The new Modified Cream Ring Test is being evaluated in this laboratory and five field laboratories by comparison with epidemiologic, serologic, and cultural studies of animals in cream ring test positive and negative herds. Results to date appear to support the developmental work in which it was observed that the Modified Cream Ring test would detect up to 50% additional samples containing brucella agglutinins in those cream samples which were partially deteriorated or sour.

Studies of problem herds are continuing using nine serological tests of serum and milk supplemented by bacteriologic and fluorescent antibody studies to isolate or identify the organism from those animals which are slaughtered. With the aid of these procedures 20 counties in Minnesota have been certified as brucellosis-free, and the studies are progressing in approximately 50 other counties. It has been interesting to note that in the first 18 counties certified - some for several years - only 5 herds have been found to have reinfection.

Studies of the milk brucella ring test are continuing to obtain more information regarding optimum methods of storage, handling, and preserving composite milk samples collected for Babcock testing in the plants in order that optimum samples for BRT testing may be obtained from these composite samples. Studies are now in progress to determine the effect of different concentrations of mercuric chloride on brucella agglutinins in the milk of cows at an earlier stage of infection. Studies are being conducted using 1½ ml. and 2 ml. of milk along with the regular milk Brucella ring test. These are being accompanied by studies of herd size and the effect of the number of animals contributing to the pool of milk on the present sensitivity of the brucella ring test under Minnesota conditions. These studies are particularly pertinent since the size of Minnesota herds is increasing each year.

In 1961, the University of Wisconsin, under a cooperative agreement with the USDA, developed a culture system using guinea pig monocytes for the assay of toxicity and antigenicity of extracts isolated from cells of B. abortus. The basic characteristics of a strain of brucella phage, useful in taxonomic work, have been described.

In 1962, at the University of Wisconsin, in studies of 262 herds with long standing infection, 230 were freed of brucellosis by a combination of serologic tests. In these herds the combination of plate tube and whey agglutination tests properly identified 41 of 48 shedders. The tube test alone identified 40 and the plate test alone only 25. The discrepancy between plate and tube test results on infected animals could be reduced by half but not eliminated by incubating plate tests for 15 minutes.

Two hundred forty-nine herds with consistently positive ring tests but without reactors to the plate test have been studied. In most cases, a single animal has been shown by titration with pooled negative milk in a ring test, to be responsible. Of 49 such animals which were subjected to bacteriologic examination, 18 were shedders. All were properly identified by the combined whey and tube tests.

Fractions of Brucella abortus have been studied in a culture system, using guinea pig monocytes. Differences have been found in toxic and hypersensitive effects of various fractions.

A phenotypic morphological effect of brucellaphage on some strains of B. abortus has been shown to be due to a carrier state.

B. Paratuberculosis (Johne's Disease)

In 1961 research was continued at the Animal Disease and Parasite Research Division's Station at Auburn, Alabama, on a herd of cattle infected with Johne's disease which had been under study for 56 months. During this period, 94 animals have been culled and examined post-mortem. Neither the complement-fixation test nor the hemagglutination test can reliably distinguish between infected animals and non-infected animals as determined by postmortem results. Although the skin test is commonly used as a diagnostic test, results thus far obtained in this herd indicate that it is far from satisfactory.

An experiment designed to study the use of a vaccine against Johne's disease in sheep has been in progress 4 years. Postmortem examinations have been conducted on 25 vaccinates and 31 controls. A few typical small acid-fast bacilli were observed in the intestinal mucosa of 2 vaccinates and 1 vaccinate was destroyed in an advanced stage of the disease. Two controls died from Johne's disease, 8 others were destroyed in advanced stages of the disease, and 2 animals randomly selected were found to be moderately infected on post-mortem. From these results it appears that the vaccine has definite value as a control measure for Johne's disease.

Four rabbits were inoculated intravenously with Mycobacterium paratuberculosis obtained by digesting infected intestinal mucosa with trypsin. These rabbits all remained healthy and in 3 instances typical small acid-fast bacilli were observed in smears prepared from intestinal mucosa. However, no evidence of progressive Johne's disease was observed in any of the rabbits.

Primary cultivation of M. paratuberculosis from infected tissues was best accomplished by trypsin digestion of intestinal mucosa, followed by treatment with NaOH, and inoculation of lymph gland-egg yolk medium. It was found that the retarded growth exhibited by organisms from infected lymph glands might have been due to antibodies present in the lymph gland tissue. Growth of a recently isolated strain of M. paratuberculosis was not inhibited by 10,000 units of penicillin when the organisms were seeded on lymph gland-egg yolk medium, but there was no growth on lymph gland medium.

Protoplasm from M. paratuberculosis was used as an antigen for antibody production and as an antigen in precipitin tests. When it was used to elicit antibodies it produced a nodule at the site of inoculation and the animal produced precipitating antibodies within 2 weeks, but it did not react to intradermic johnin. When the protoplasm was used as a precipitin antigen, it reacted strongly with the serum from a sheep with clinical Johne's disease, and weakly with sera from sheep vaccinated with whole M. paratuberculosis or exposed to infection with Johne's disease but not showing clinical symptoms.

In 1962 the work on paratuberculosis was moved to the National Animal Disease Laboratory, Ames, Iowa. The herd of cattle ranging in numbers from 161-195 animals in which Johne's disease has been a severe economic problem has been under study for 68 months. During this period, 119 animals have been culled and examined postmortem. One of the 25 that were culled during the past 12 months showed clinical signs of Johne's disease. Neither the complement-fixation test nor the hemagglutination test can reliably distinguish between infected animals and noninfected animals as determined by postmortem results because M. paratuberculosis were found in 25% of the cattle negative to the complement-fixation test and in 36% of the cattle negative to the hemmaglutination test. The skin test using intradermic johnin is commonly used as a diagnostic test, however, results thus far obtained with intradermic johnin in this herd indicates that it needs much improvement because M. paratuberculosis were found in 40% of the non-reactors examined postmortem.

Two experimental skin test products were also tried on this herd. One was almost equal to standard johnin in potency and the other was about 2/3 as potent.

Acid-fast bacilli were demonstrated in the lungs, spleens, kidneys and livers of rabbits for at least eight weeks after they had been injected intravenously with either killed or live M. paratuberculosis. Therefore, it is questionable whether a true infection was established in rabbits with the live microorganisms.

Continuing studies of various types of media showed that lymph node-egg yolk medium is better than simple egg yolk or lymph node media for primary isolation of M. paratuberculosis. A medium prepared from infected intestinal mucosa produced luxuriant growth of M. paratuberculosis after prolonged incubation.

Rabbits and guinea pigs were vaccinated with a protoplasmic antigen and the controls with heat-killed organisms. They were skin tested with johnin and tuberculin. The skin reactions due to protoplasmic vaccine were small or absent. Those due to vaccination with heat-killed organisms were very large. Gel-precipitin tests with sera from protoplasm vaccinated rabbits produced only one definite precipitation zone. No precipitation zones were produced by sera from rabbits vaccinated with heat-killed organisms.

A new proteolytic enzyme (X-108) prepared by American Cyanamid Company, was found to be better than trypsin for digestion of tissues infected with Mycobacterium paratuberculosis and liberation of the bacilli.

C. Vibriosis

In 1961 the studies on vibriosis were in progress at the Animal Disease Station in Beltsville, Maryland. The morphology and biochemistry of bovine Vibrio strains isolated from 87 cattle in a survey of 56 herds were studied. The V. fetus strains (pathogenic) were separated into 2 major types and a subtype of Type 1, by colony dissociation and biochemical reactions. Type 1 strains grew fastidiously and growth was enhanced by the addition of glutathione and sodium thioglycollate to the medium. Both smooth and smooth-cut glass colonies were observed in primary cultures. Type 2 was characterized by more abundant growth and numerous stable variant colonies. Both smooth and rough colonies were observed in primary cultures. Subtype 1 strains grew moderately in culture mediums. Cut glass colonies were observed only at primary isolation. Vibrio bubulus strains (nonpathogenic) grew well on blood agar but they did not adapt easily to Albimi agar. Smooth and granular colonies were observed in primary cultures. Biochemical tests which were made on cultures of smooth and variant colonial forms failed to show any change in activity due to dissociation.

Bulls that were infected with Type 1 and Subtype 1 strains of V. fetus infected all heifers bred to them. The infected heifers became repeat breeders. However, bulls that were infected with Type 2 V. fetus infected only 1 of 12 heifers by breeding, 11 of which required only 1.4 services per pregnancy. Vibrio which was biochemically similar to the Type 2 exposure strain was isolated from both the vagina and the rectum of 1 heifer. This heifer was apparently not pregnant at slaughter 21 days after service.

A bull that was infected with Type 1 V. fetus infected 12 heifers at their first service. Uterine infection was found in 9 of these heifers at necropsy. A neutrophilic and lymphocytic infiltration of the endometrium was found on histopathologic examination. In contrast, neither infection nor histopathologic changes were observed in 5 virgin heifers that were necropsied at intervals during the estrus cycle. Seven heifers that were bred to a noninfected bull became pregnant, but all were free of histopathologic changes at necropsy.

Cultures of the three types of V. fetus and fecal samples from cows infected with Type 2 V. fetus, were given per orum to noninfected cows. Isolations of Type 2 V. fetus were made from 21 of 60 post-exposure fecal samples. At necropsy, Type 2 V. fetus was isolated from the intestinal tract from 3 of the 7 animals and also from the bile of one cow.

In 1962, most of the work on vibriosis was done at the National Animal Disease Laboratory at Ames, Iowa. Comparative infectivity studies of 20 strains of V. fetus included 12 strains of type 1, 5 strains of subtype 1, and 3 strains of type 2. Only type 2 strains consistently infected the gall bladder and

duodenum of mice, guinea pigs, and rabbits. These results compare favorably with experiments in cattle in which only type 2 V. fetus was recovered from the gall bladder and/or duodenum. It was concluded, therefore, that the digestive organs may harbor virulent V. fetus intestinalis and remain the source of infection in cases of sporadic abortion in cattle.

Chemical, serological, and toxicological analyses were made on cell wall, intracellular, and extracellular fractions of the organism. The cell wall is composed of a complex group of amino acids characteristic of Gram negative organisms. At least 3 antigen-antibody complexes were found in agar-gel diffusion tests. Only the intracellular fraction was lethal for mice in the preliminary studies made.

A liquid culture medium was developed in cooperation with the chemical and physical investigations which improved the yield of V. fetus cells from 0.5 gram to 2.0 grams per liter of culture medium.

In 1961, at the New York State Veterinary College, Ithaca, New York, under a cooperative agreement with the USDA, experiments were conducted to determine if vibriosis was spread by the methods commonly used for semen collection in an artificial insemination stud. Thirty-one bulls were used - half were collected in the usual manner and half with a so-called strict collecticn technic with no false mounts. Over a 2-year period, no new cases of vibriosis have been detected in the strict collection group, whereas 2 new cases were observed in the regular group. The strict collection method appears to minimize the chances of spread of the infection.

Eight bulls were treated by the application of a 1% furoxone ointment to the penis and sheath. Ten bulls were treated with the same ointment plus a 1% furaltadone solution in a series of three treatments at 47-72 hour intervals. Seven bulls, following treatment, were found to be free of V. fetus at the end of the experiment. The procedures are being continued using the same chemicals in a more absorbable base.

Attempts to find more suitable culture media and technics are being continued. Albamycin has proved highly useful to suppress the growth of B. proteus. The filtration technic of Plummer and associates is being used regularly with highly encouraging results. In the filtered material only 2 percent of the cultures were overgrown, whereas 73 percent were overgrown when non-filtered material was used.

D. Tuberculosis

In 1961, research workers at the ADP Station at Auburn, Alabama, reported finding a herd consisting of 200 cattle, all thought to be free of Johne's disease and tuberculosis, disclosed 24 animals with reactions to tuberculin and/or johnin. Three of these were calves. The 21 adults were slaughtered, examined post-mortem for tuberculosis (none was found) and material was obtained from each animal which was processed and inoculated onto medium and

into small laboratory animals. None of the laboratory animals developed lesions of tuberculosis or reacted to the tuberculin test. However, acid-fast bacilli were obtained from cultures from 3 animals. These have not been identified. The ileocecal valves and adjacent regions of the intestinal tract were examined for the presence of bacilli resembling Mycobacterium paratuberculosis. Such organisms were found in the intestines of 3 animals. Material from these three specimens has also been cultured, but this work has not yet been completed. Titers of 1/10 or more were observed in serums of 15 animals prior to skin testing and this titer remained fairly constant each week for four weeks.

In 1962, at the National Animal Disease Laboratory, at Ames, Iowa, data were collected on the response in cattle to intradermal cervical tests with different strengths of tuberculin given in 0.1 ml. amounts. The NADL herd was tested on the assumption that the animals were free of tuberculosis based on a history of no tuberculosis in the herd for over 25 years.

The skin reactions were recorded in terms of increases over and above the original skin thickness. The reactions ranged from (pp) a pin point disturbance through (p^1) 2 mm. increase, (p^2) 3 mm. increase, (p^3) 4-5 mm. increase, and (p^4) 5-6 mm. increase. In general, a p^2 or greater is considered a significant reaction.

The data showed that when the animals were tested with ARS tuberculin diluted to 10% of the regular strength supplied for use in the field, no animals had greater than pp reactions at 48 hours and only 3 of 120 animals had p^1 responses at 72 hours. In contrast, testing the same animals with regular strength tuberculin resulted in 14 p^1 and 3 p^2 reactions at 48 hours. Ten p^1 and 3 p^2 reactions were found at 72 hours. Testing 69 of the animals with tuberculin concentrated to 4 times the regular strength did not give results differing greatly from those occurring with regular tuberculin, although 1 p^3 and 1 p^4 reaction was observed at 48 hours and 2 p^3 reactions at 72 hours.

The evidence indicates that a less concentrated tuberculin results in fewer skin reactions of all sizes in a herd apparently free of tuberculosis.

As part of a cooperative research problem with Chemical and Physical Investigations, a comparison of the antigens in BCG culture filtrate, water extracts of BCG, and preparations from unextracted and extracted BCG subsequently broken under mechanical pressure, have been compared. By gel-precipitin techniques, common antigens were demonstrated in all of the preparations. The cracked cell preparations had an additional precipitin line not demonstrated with the other antigens.

In 1961 and 1962, at Michigan State University, East Lansing, under a contract with the USDA, investigations were continued on the cause or causes of no-gross-lesion tuberculin reactors and to improve methods of diagnosis of bovine

tuberculosis. All acid-fast microorganisms isolated by various methods from animal tissues, including skin lesions, and soil samples were identified insofar as possible by selected morphologic, cytochemical, infectivity and sensitizing characteristics. Emphasis was placed upon there being no single test which conclusively identifies any one species of the known mycobacteria. For instance, Mycobacterium tuberculosis produces nicotinic acid and this is perhaps the most dependable and widely used characteristic for confirmation. However, M. microti and M. ulcerans are also positive. The demonstration of the tubercle bacilli in pathological material is the only sure method for diagnosis.

A wide spectrum of acid-fast organisms, including many atypicals with varying degrees of virulence and all reacting to some extent to mammalian, avian and to various purified protein derivatives (PPD), were found in the tissues of tubercular positive cattle. To date mycobacteria isolated from 263 cases have been tentatively identified. Of these, 81 were from bovine tissues, other than skin lesions, 22 being M. bovis and 2 M. avian; 85 from skin lesions, 5 from bovine semen specimens which may have become contaminated at time of collection, 96 from swine lymph nodes, 1 being M. bovis, 35 from soil and barn samples, 5 from feed samples and 1 each of guppy and mink origin. In some instances a number of isolants from a single case were tested.

The isolation of many acid-fast organisms, which may be incorrectly called "atypicals", from tuberculin positive cattle that are infective to laboratory animals reveals for the first time a new group of organisms that must be fully evaluated by large animal studies. Infectivity, sensitivity, transmissibility and serologic and immunologic behaviors must be investigated before these organisms can be cataloged and their importance in disease control evaluated.

Research findings that the procedures used for the isolation of acid-fast organisms from samples containing low populations is applicable for the isolations from samples containing high populations. However, the reverse is not true. A total of 121 cases consisting of tissue samples were each processed by two methods, the results reported show there were 22 positives isolated by the sodium hydroxide method and 44 isolates by the pentane-digest method.

The difficulties encountered in the classification of acid-fast organisms isolated from gross lesion and no-gross-lesion reactors and the increasing interest in the pathogenic significance of unclassified acid-fast bacteria have led to the initiation of research concerned with the nature of proteins in and produced by various types of acid-fast organisms. The primary purpose of current studies is to develop procedures to produce more specific sensitins for the identification and differentiation of the various mycobacteria and mycobacterial infections in animals.

E. Mucosal-Respiratory Disease-Complex.

In 1961 the studies conducted at Purdue University, Lafayette, Indiana, under a cooperative agreement with the USDA on reciprocal cross protection tests in calves indicated the Indiana virus diarrhea agent (IVD), the Oregon virus diarrhea agent (OVD), and the Indiana mucosal disease agent (IMD) were immunologically identical. Attempts were made to neutralize the cytopathogenic OVD agent with 14 different types of antiserum. Seven types related to the mucosal-disease virus-diarrhea complex did neutralize this agent the others did not. Various determinations were made of the properties of the OVD agent. It was found that it contained little or no lipid, was between 50 and 100 millimicrons in size, did not cause hemadsorption of guinea pig erythrocytes, and was destroyed by 56 C. in approximately 30 minutes.

The OVD agent did not readily adapt to embryonated eggs, suckling mice, or rats. Sheep fetuses propagated the agent in low concentrations following in utero inoculation.

A specific pathogen free cattle herd was originated by performing 22 caesarean sections and raising the calves in an isolated environment.

Other results indicated that several of the mucosal disease and virus diarrhea agents are related. This provides evidence that these agents are actually part of the etiology of the diseases and indicates that virus diarrhea and mucosal disease may be different clinical manifestations of the same disease. This hypothesis will be difficult to prove until means are devised to produce the typical clinical syndromes.

Calves contact-exposed to inoculated sheep developed signs of disease. When these calves were challenged with virulent blood they developed typical signs of experimental virus diarrhea. Blood collected from the sheep during the period of leukopenia was infective for calves, indicating that viremia existed at the time sheep-calf contact was made. Calves infected with the sheep blood resisted challenge with virulent bovine blood.

Attempts to demonstrate calf-to-calf contact transmission were unsuccessful. Calves exposed to virus by the nasal-oral route did not develop signs of disease. When these calves, and calves in contact exposure with them, were challenged with bovine virus they all reacted in a typical manner. The contact calf exposed to an intravenously inoculated pen-mate did not develop signs of disease. It was fully susceptible when later challenged.

In 1962, at Purdue University under a cooperative agreement, investigations of field outbreaks in the mucosal disease complex continue to reveal a sporadic distribution of both MD and VD. Chronic diarrheal syndromes in feeder calves continue to pose a diagnostic problem for practicing veterinarians in Indiana.

There is usually no history of an acute disease syndrome (such as acute virus diarrhea) in these chronically affected herds. Invariably only a portion of the herd is chronically affected. It is probable that affected herds experienced a previous, mild infection of short duration, although this information is not always obtained from the herd history. In these cases, diagnosis of VD-MD complex is made on a basis of history (when available), clinical observation, and serology. Serological diagnosis is based on high titers of neutralizing antibody against the type strain of VD virus--Oregon C24v.

In three trials, intravenously inoculated sheep were placed in direct pen-contact with susceptible calves. Control transmission tests with calves were performed in which orally and parenterally inoculated calves were placed in direct contact with other susceptible calves. Bovine blood was used as stock virus. In no case was contact infection established in calves exposed to infected sheep. When later challenged, these calves were fully susceptible to the virus.

Calves in direct contact with other calves given virus either naso-orally or parenterally failed to show signs of experimental infection. Subsequent challenge with stock virus showed that contact-exposed calves were fully susceptible.

Although viremia was demonstrated in sheep in two trials, exposure of calves to them at this time did not result in contact infection.

Tissue culture systems employing bovine and ovine thyroid tissues were developed. Preliminary studies indicate that Oregon (C24v) and Nebraska (M-833) agents replicate and produce cytopathic effects in monolayers of ovine thyroid cells. This cell system is applicable for virus neutralization tests. Studies are in progress to determine the susceptibility of these cell systems to virus isolates that fail to elicit cytopathic effects in other cell lines.

Procurement of over 40 calves by Caesarean section furthered development of the Specific Pathogen-Free (SPF) cattle herd. The opportunity was taken to study the possible presence of bacteria and viruses in the in utero environment. Bacteriological examination of the fetus, umbilical cord blood, and extra-embryonic fluids showed that these calves were apparently free of bacteria at time of section. Study of developmental changes in blood cellular elements and serum proteins were made at birth and early in post-natal life. Methods for rearing of these colostrum-deprived calves were refined.

Sterile swabs were used to obtain specimens from the amino-allantoic fluids, fetal, skin, anus, and nose. Samples of placental blood have been obtained from the umbilical cord. All samples were subjected to anaerobic and aerobic culture conditions. Blood agar and thioglycollate media were inoculated and incubated at 37 C for 48 hours.

Bacteria were present in cultures from four of 29 calves. Two isolates of non-pathogenic organisms were from the nasal area. In both calves the specimens were not obtained until after respirations had been initiated and the isolates were considered to be air borne contaminates. A gram positive cocci from the amniotic fluids and a coliform organism were isolated from two other calves. Possible contamination of a calf was known to result from an accidental perforation of the maternal intestinal tract. The efficiency of the bacteriological procedures was demonstrated by the isolation of bacteria from the nose, skin, and anal region of this calf.

The bovine placenta effectively interferes with the transfer of maternal antibodies to the fetus. Passive immunity is provided for the newborn calf by enteric absorption of colostral antibodies. As the Caesarean derived calves were deprived of colostrum, this provided an opportunity to evaluate the functional development of the reticulo-endothelial system of the calf. Serum or plasma samples were collected from the blood samples obtained for hematology. Electrophoretic analysis of the serum (plasma) proteins are being utilized to give a qualitative evaluation of the various components, which are then quantitated by nitrogen determinations. This work is in progress.

In 1961, at the Iowa State University, Ames, Iowa, under a cooperative agreement with the USDA, it was found that, with the exception of the combination of the Sanders and PPLO agents and the delayed combination of Sanders and Nebraska MD agents, the series of experiments involving a combination of agents of the mucosal disease viral diarrhea complex produced a response which closely approaches the field syndrome than if these agents are inoculated by themselves. On the basis of these preliminary experiments one can hypothesize that mucosal disease as it is seen in the field probably is the result of an infection by a combination of specific agents plus possibly other factors. The proof or disproof of this hypothesis awaits further trials.

A definite, repeatable syndrome can be reproduced in calves by the inoculation of the Sanders agent. Furthermore, it has been observed that this agent can be serially passed from one calf to another with the typical syndrome appearing at each passage. Swine and goats showed no conclusive response to the Sanders agent. Sheep appear to be susceptible to the Sanders agent and develop a syndrome milder but similar to that seen in calves. Present information indicates that mice, guinea pigs and chicken embryos show no evidence of susceptibility to the Sanders agent.

In 1962 workers at the Iowa State University reported a viral agent was isolated from a calf from the Colglazier herd showing typical signs of mucosal disease. This agent was recovered from deep scrapings of involved Peyer's patches. Isolation was accomplished by four passages through primary cell cultures of bovine kidney and testicle cells. The virus produces distinct cytopathic effects in primary cell cultures of bovine origin. A plaque method was devised enabling a highly accurate titration method. Using this method,

cross neutralization tests against known strains of virus diarrhea agents were done. Results of these tests indicated the absence of any serological relationship between the newly isolated agent and two reference VDV virus antisera produced in rabbits. The virus is not pathogenic for mice by any route. There is indication that a disease syndrome is produced in cattle, although insufficient trials will not permit us to make definite claims.

Preliminary trials using fluorescent antibody techniques to detect specific viral antigen in cell cultures and frozen sections have been done. It appears that specific antigen can be localized in cell cultures. We are presently trying to improve our methods with the intention of applying this method to diagnosis and basic research including the study of non-cytopathic strains and their serological relationships.

Attempts to isolate enteroviruses from 128 animals located in eight different herds and ranging in age from 3 days to approximately 5 years have been negative. Serum samples collected from 37 animals either affected with mucosal disease or located in infected herds have failed to neutralize the Nebraska mucosal disease agent. Serum samples from experimentally infected calves or hyperimmunized rabbits have shown that only the Nebraska and the North Dakota agent antiserum are capable of neutralizing the Nebraska mucosal disease agent. Antiserum against the Sanders, Merrell, Indiana virus Diarrhea, C-80-K and C-24-V agents fails to neutralize the Nebraska mucosal disease agent.

Many combinations of agents isolated from the mucosal disease-virus diarrhea complex have been used in an attempt to reproduce a typical case of mucosal disease terminating in death. However, our experimental trials have not been successful in this regard.

In 1961, the University of California, at Davis, California, in cooperation with the USDA, reported a filterable agent, which appears to be viral in nature, has been isolated from two different cows out of some 30 cows clinically diagnosed as suffering from bovine lymphosarcoma.

Cattle experimentally infected with infectious bovine rhinotracheitis (IBR) virus showed detectable antibodies after 44 months. Studies to date have been encouraging enough that a CF serology test may be devised to detect IBR antibodies.

A preliminary survey by means of serum neutralization in tissue culture has been initiated in the comparison of some cattle diseases of the United States to those which exist in Germany. Attempt to isolate the virus diarrhea virus of cattle in tissue culture from materials obtained from clinically diagnosed cases of virus diarrhea was not successful. Studies with the "viral" agent of bovine abortion were made in regard to its behavior in young calves and possible placental transmission.

In 1961 the Colorado State University, at Fort Collins, under a cooperative agreement with the USDA, reported that twenty cattle were continued on an experiment in a large animal virus isolation laboratory at Fort Collins to determine the rate of decline of antibody titer following inoculation with virulent virus of infectious bovine rhinotracheitis. During the year 3 animals showed 1000 fold reductions of antibody titers after 7 to 10 months. Their immunity was challenged by intratracheal inoculation of virulent virus. One developed typical symptoms of the disease, 2 showed increase in body temperature only, and 3 showed anamnestic response by augmentation of titers. The remaining 17, with the exception of the 4 controls, still had high titers 18 months after intratracheal, intramuscular, or intravenous inoculations. Seemingly there is no significant difference in titers as a result of different route of inoculation.

By the use of the fluorescent antigen-antibody technique, a study of virus infected bovine kidney cells in a tissue culture system showed no evidence of virus aggregation forming the inclusion body. Materials were received from 48 outbreaks for virus isolations from 11 States and from 280 cases for serum neutralization tests.

In 1962 studies were continued at the Colorado State University under the cooperative agreement, and during the year the serum neutralizing titers of cattle which are kept in the isolation units, did not show significant reduction. There was no evidence of difference in neutralizing antibody level between the two groups of animals infected intratracheally and those infected intramuscularly.

In a study of abortion of cows in relation to IBR infection, the isolation of IBR virus from aborted fetuses was the first stage of the work. Field reports of abortions were investigated. Fetuses were collected, and different organs of the fetus were used for isolation of IBR virus. The virus was isolated from the lung tissue of the fetus only. If more fetuses could be obtained it might be possible to isolate the virus from other organs.

In studying IBR epizootics in ranch cattle, fifty animals (47 deer from 1 to 6 years of age, and 3 elk from 1 to 2 years of age) were gathered together for testing. Blood samples from these animals were collected. These animals will be challenged or infected with IBR virus to determine their susceptibility and what sign(s) of sickness, if any, are manifested. Since deer, elk, and cattle cohabitate in pasture and range areas, the susceptibility of deer and elk to IBR may be an important factor in determining transmission of the disease.

In 1962, at the National Animal Disease Laboratory at Ames, Iowa, naturally occurring cases of BVD or MD have been studied in the NADL herd from which one agent was recovered and in one farm herd from which 4 probably identical agents were recovered. Inoculation of the NADL agent into an experimental animal was studied for development of clinical signs, blood changes, virus recovery, and production of neutralizing antibody. Periodic bleedings have

been made from the NADL herd as well as from the farm herd. Isolation of additional agents from the respective herds was attempted by making rectal swabs and leucocyte cultures from apparently normal cattle. This resulted in the isolation of an agent from 1 out of 5 rectal swabs taken in the farm herd and from 12 out of 19 rectal swabs representing all cattle in one NADL barn. In addition, 5 isolates were obtained from leucocyte cultures out of the 19 apparently normal cattle housed in the NADL barn.

Virus neutralization studies indicate that the agents isolated from rectal swabs are antigenically different from those isolated by leucocyte cultures. They also appear distinct on the basis of rapidity and type of CPE produced in tissue cultures.

Five viral agents identified with bovine virus diarrhea (BVD) or mucosal disease (MD) have been obtained from other laboratories for storage in the repository and subsequent use as reference material. The Oregon virus diarrhea agent (C24V) has been selected as the prototype since it is the one most commonly used in other laboratories.

A bovine kidney cell line designated National Laboratory Bovine Kidney (NLBK) was developed as a laboratory tool. It appears to be very useful because it is susceptible to the prototype virus as well as several other viruses of bovine origin.

F. Mastitis

In 1961 research was continued at the Animal Disease Station at Beltsville, Maryland, on the development of a medium for the titration of lactenin. Beef infusion, when added to a medium containing casin hydrolysate, vitamins, glucose, and various salts, permitted the growth of Streptococcus pyogenes. The growth factor will pass through a cellophane membrane, will migrate to the cathode chamber in an electrodialysis cell and is stabile to autoclaving or concentration at 80 C. in a flash evaporator. The growth factor is not associated with carbohydrates or minerals present in the ash. Several amino acids, purines and a pyrimidine have been tentatively identified by chromatographic methods but have not been related, as yet, to the growth factor.

In 1962, at the National Animal Disease Laboratory, Ames, Iowa, research on mastitis was continued on the study of factors in milk which inhibit the growth of Streptococcus agalactiae. The inhibitory activity of milk was not affected by preparing whey by treatment with acid or rennin and centrifuging the wheys at speeds up to 17,000 rpm (34,800 X C). No loss in inhibitory activity was caused by dialyzing whey against de-ionized water or a 0.02 M phosphate buffer of pH 7.0 for 48 hours at 5°C. However, electro-dialyzing whey at room temperature for 7 hours at constant current (100 m.a.) resulted in a decrease in activity of at least 50 percent.

When milk from infected quarters was titrated raw, the dose-response curve typical for the inhibitory activity of raw milk from non-infected quarters was altered because of increased acid production. This increase in acid production was eliminated by pasteurizing the milk from the infected quarters before titration.

Research was also continued on the development of a culture medium of defined composition to grow streptococci of serological Groups A and B for use in assaying the growth inhibitory factors in milk. A culture medium, composed entirely of known constituents except for 1 mg per milliliter of crude egg albumin will support good growth of Streptococcus pyogenes. Washed cell suspensions which were grown in Difco brain-heart-infusion broth were used as a source of inoculum. Purified egg white fractions such as conalbumin, ovalbumin and perhaps lysozyme will substitute for the crude egg albumin. This is the same basic medium used for microbiological assay of amino acids and is described in Cornell Experiment Station Bulletin No. 337, 1955.

The same medium without the egg albumin, supported good growth of several cultures of Streptococcus agalactiae. These organisms are less fastidious than the group A cultures and will grow in a medium in which all components are known.

Growth of these two groups of organisms can be measured with speed and accuracy by titration of the acid formed. Both groups converted 90 or more per cent of the glucose fermented to lactic acid.

Four strains of Str. agalactiae were shown by analysis of their fermentation products to be homofermentative lactic acid bacteria.

In 1961, the University of California, at Davis, in cooperation with the USDA, in studies in a limited number of animals, the following results may be of significant value for future studies on the big and important research problem of mastitis.

A small number of Aerobacter aerogenes may induce clinical mastitis in a normal mammary gland. Repeated small doses did not lead to an intensification of the clinical response but may have contributed to development of an infection of 20 days duration.

A pre-existing chronic inflammation caused by Ps. aeruginosa gave some protection against development of an acute clinical phase of mastitis following the introduction of 4,000,000 A. aerogenes. A persistent infection was not established for the longest period of residence in any gland was 9 days.

In an attempt to counteract A. aerogenes infection, the inoculation of extremely large numbers of micrococci led to a high and somewhat persistent level of leukocytosis accompanied by swelling and clots. This was followed in 5 days by disappearance of the A. aerogenes infection, and on the 6th day the micrococci disappeared.

The introduction of approximately 1 million dead A. aerogenes organisms into normal lactating mammary quarters induced a leukocytosis of 4 days duration with the peak number of cells in foremilk reaching 4.0 to 5.0 million/ml. Viable organism in the same dose level stimulated cellular responses in 8½ hours of 60.0 million cells/ml and in addition swelling of the gland was observed for 3½ days and rectal temperature reached 106.0°F at 8½ but quickly returned to normal. During the period of persistence of the A. aerogenes within the gland a see-saw pattern of cellular activity was seen with two or more peaks of considerable magnitude followed immediately by a rapid fall in cell numbers. Presence of A. aerogenes in the milk was demonstrated mostly only by incubation of the milk before culturing on the surface of blood agar plates.

The information gained from other limited clinical, bacterial, and chemical studies on mastitis points to the value of gathering data during the early phases of the response to bacterial infection in the mammary gland in order to better understand the natural mechanisms for defense of the total animal. The response of the animal to infection is immediate and, therefore, future studies should include examinations at frequent intervals during the first few hours.

In 1962, the University of California, cooperatively with the USDA, studied a culture of Aerobacter aerogenes originally isolated from the bovine udder in 1959 which was employed in the production of experimental mastitis. It was demonstrated that less than 100 organisms, when introduced into the teat and gland cistern of an absolutely normal lactating mammary gland, are capable of inducing in 10-15 hours an acute mastitis with an associated systemic reaction. It was also clearly demonstrated that a pre-existing leukocytosis, even of low order, has significant protective benefits. Systemic signs, such as elevated body temperature and anorexia, and local signs, such as detectable swelling of the inoculated gland, are prevented from developing when leukocytes are present in the udder secretion at the time of introduction of the culture. The inoculum, however, causes an increase in exudation of neutrophils so that for several milkings the cell count/ml. is increased significantly, although visible signs such as clots in the milk may not occur.

The experiments reported here strongly indicate that mammary glands must be completely free of even a minimal inflammatory reaction before coliform-type organisms are able to produce acute mastitis. Patency of teat canal is also involved. The most apt to have patent streak canals are the older animals and since the older animals most regularly have a leukocytosis from repeated udder stress,such cows at the same time are protected against coliform mastitis. Application of extensive intramammary therapy to such cows may produce complete freedom of such glands from leukocytosis and render the animals susceptible to coliform mastitis.

G. Shipping Fever

In 1961, at the Animal Disease Station, Beltsville, Maryland, as a continuation of studies on the etiology and transmission of shipping fever, 3 Holstein calves were injected intramuscularly with live para-influenza 3 virus, three with killed Pasteurella multocida and P. haemolytica, and three with a combination of the live virus and killed Pasteurella spp. Para-influenza was not transmitted from the injected calves to contact controls as determined by hemagglutination-inhibition test and attempts at virus isolation.

The 9 injected and 5 control Holstein calves were assembled in a feed lot with 27 Angus calves, of which several were showing clinical signs of shipping fever. P. multocida, P. haemolytica, and para-influenza 3 virus were transmitted to the Holstein calves by contact. P. multocida, P. haemolytica and para-influenza 3 virus were isolated from 8, 13, and 9 Holstein calves respectively. The injection of the above three agents before contact exposure could not be adequately evaluated under the conditions of the experiment.

In 1962, at the National Animal Disease Station, Ames, Iowa, fundamental studies on the nutrition and metabolism of Pasteurella sp., believed to have etiological relationship to shipping fever, are in progress. Blood agar base containing hemin was the best medium tested for the enumeration of P. multocida and P. hemolytica, producing the highest, least variable counts, while gelatin-saline was the best diluent for counting procedures.

Studies designed to investigate experimental infection produced by para-influenza-3 (SF-4) virus in laboratory animals and in various tissue culture systems are in progress. Parainfluenza-3 virus multiplies readily in the amniotic sac of 13- to 14-day-old chick embryos, before and after serial passage, without losing its characteristic pathogenicity for primary cultures of embryonic bovine-kidney cells. Infection was not maintained by serial passage of egg or tissue culture virus in the allantoic cavity of younger embryos. Attempts to demonstrate plaque formation by parainfluenza-3 virus in primary monolayer cultures of embryonic bovine-kidney cells with agar overlays were not successful.

H. Leptospirosis.

In 1961, at the Animal Disease Station at Beltsville, Leptospira pomona was grown in a medium in which the whole serum had been replaced by 1% albumin. Vitamin B_{12} was required for growth. Ammonia may be a major source of nitrogen. No change in antigenic characteristics occurred.

Anaplasmosis infection did not protect cattle from infection with Leptospira pomona. Stress from leptospirosis triggered recurrences of anaplasmosis.

Leptospira pomona infection was fatal to chinchillas in as early as 6 days. Two animals inoculated with bovine kidney tissue from which Leptospira pomona was isolated by culture failed to become infected although they were susceptible to infection upon subsequent challenge.

No new infections have occurred in the Jeanerette Dairy herd where a long term vaccination study is in progress.

In 1962, at the National Animal Disease Laboratory, Ames, Iowa, Leptospira pomona and 11 other major serotypes have been successfully cultured through an indefinite number of transfers in a medium whose major and most critical components are bovine albumin (Dubos oleic albumin complex DIFCO, Detroit, Michigan), Vitamin B-12, and ammonium chloride.

Maximum growth is achieved at a concentration of 1 percent albumin, although continuous subculture is possible at 0.25 percent albumin. Vitamin B-12 is the only vitamin which has been shown to be an absolute vitamin requirement in this specific medium. This need can best be demonstrated after subculture in the absence of added culture medium B-12. The amount of B-12 bound to the albumin has not been determined. No requirement for thiamin in this medium could be demonstrated. Consequently, thiamin has been deleted from the growth medium.

The ammonium ion source of choice is ammonium chloride. It is stimulatory at low levels, nontoxic at high levels, and cannot be replaced with monovalent cations. The deletion of ammonium ion still allows for continued growth in the presence of albumin.

Magnesium ion was found stimulatory to growth, but is not an absolute requirement for growth in the presence of the protein albumin. The stimulatory activity is not replaceable with divalent cations. The optimal level of sodium chloride in this medium is in the range of 0.27 - 0.37 percent.

The absolute need for trace metals is still in question and may remain so until deletion of the protein is achieved. The same is true of l-cystine.

Minute inocula of the order of .25 percent as opposed to conventional 10 percent inocula will attain maximal cell crops during extended incubation periods in the current medium.

The growth supporting activity of the medium is not diminished by temperatures of 56° to 70° C. Complete destruction of growth supporting activity is seen at 82° to 84° C. after 30 minutes exposure.

This medium has been prepared in semi-solid form containing 0.2 percent regular agar and permits maintenance of stock cultures for prolonged periods of time.

The vaccination study in the Jeanerette dairy herd has continued. The fall 1961 bleeding was accomplished as planned but extensive personnel changes at Jeanerette interferred with the spring bleeding. To date no evidence of infection has been observed in either the vaccinated or nonvaccinated animals. The herd is gradually becoming seronegative through the coincidental removal of reactors in the routine culling process.

I. Epizootic Bovine Abortion.

In 1962, the University of California, Davis, under a cooperative agreement with the USDA, conducted investigations of epizootic bovine abortion (EBA). The studies under way include 1) attempts to ascertain whether the virus of EBA can induce abortion when administered either orally or nasally. These are believed to be the natural routes of infection. Should it be shown that the virus is capable of producing abortion when exposed to cattle through either or both of these avenues, it will remove any doubts that it might not be the sole cause of the condition. 2) attempts to determine whether cattle recovered from infection with the EBA virus are refractory to abortion when challenged with virulent virus. Such information is fundamental to any prevention program based on immunization. 3) Field trial studies to establish whether multiple injections of an inactivated EBA virus vaccine preparation will confer immunity to abortion. Contemplating that a viable agent will be needed to produce a satisfactory immunity, efforts are currently under way to attentuate the EBA virus by serial passage in tissue culture for possible use as an immunizing agent. 4) Epidemiological studies to ascertain whether the EBA virus is tick-transmitted and whether ticks are the reservoir of the virus in nature.

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AREA NO. 2 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF SWINE

Problem. Profitable swine production depends largely on the ability to control diseases. Swine diseases cause losses estimated at more than $200 million annually. In order to control and eventually eradicate these diseases, a thorough knowledge of causes, diagnostic procedures, preventive procedures, and treatments is required. Although a great deal of excellent research has been and is being accomplished, a vast amount of research is still required to obtain this knowledge. At present, the causes of several important swine diseases are unknown or incompletely understood. Extensive fundamental research on swine diseases is essential to the welfare of the swine industry.

USDA PROGRAM

The Department has a long history of swine disease research. For example, research on hog cholera was initiated in 1884. Research on this and other important swine diseases is a continuing long-term program. Modern research techniques in the areas of biochemistry, biophysics, pathology, microbiology, pharmacology, physiology, and immunology, are being applied to swine disease problems. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 22.3 professional man years. This effort is divided among sub-headings as follows:

Hog Cholera 8.1 at the National Animal Disease Laboratory, Ames, Iowa, the Florida Hog Cholera Research Station, Live Oak, Florida, and under a cooperative agreement with the University of Illinois.

Atrophic Rhinitis 4.0 at the National Animal Disease Laboratory, Ames, Iowa.

Transmissible Gastroenteritis 3.6 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with Purdue University and the University of California, and a memorandum of understanding with the University of Illinois.

Erysipelas 3.6 at the National Animal Disease Laboratory, Ames, Iowa, and under a cooperative agreement with the Department of Biochemistry, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey.

Brucellosis 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 18.1 professional man years divided among sub-headings as follows: death losses in young pigs 5.6, hog cholera 3.4, atrophic rhinitis 2.0, transmissible gastroenteritis 2.7, erysipelas 0.7, brucellosis 2.4, other diseases (salmonellosis, vesicular stomatitis, etc.) 1.3. Minnesota, Pennsylvania, Kansas, Ohio, Missouri, and Georgia are conducting studies on hog cholera. Indiana, Iowa, Nebraska, and Michigan are working on atrophic rhinitis. Illinois, Nebraska, Indiana, and Michigan are working on transmissible gastroenteritis. Indiana and Georgia are working on erysipelas. The research on death losses in baby pigs was carried out under regional research project NC-13.

Industry and other organizations. Veterinary biological and pharmaceutical companies conduct research on the development and improvement of immunizing agents, drugs and antibiotics for the treatment and prevention of swine diseases. Preventive vaccines for hog cholera, erysipelas, and leptospirosis are among the products being investigated. Drugs and antibiotics for treatment of respiratory and enteric infections are being developed. Estimated annual expenditures are approximately 50 professional man years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Hog Cholera.

In 1961, hog cholera research at the National Animal Disease Laboratory, Ames, Iowa, was conducted in the following phases:

Occurrence of hog cholera after immunization. Investigations were made to determine the cause of cholera outbreaks in swine that had been vaccinated. Seventeen field specimens were studied. Three had no virus present and 14 had virus which were classified as follows: 3 variant viruses; 9 regular viruses, and 2 immunizing viruses. Seven of the regular virus were of such low virulence that all the pigs recovered after being sick 3 to 7 days. Salmonella and Pasteurella isolated from sick farm pigs caused cholera-susceptible and experimental pigs to become sick or die when infected simultaneously with modified hog cholera vaccines.

Propagation of hog cholera virus in vitro. Hog cholera hyperimmune serums prepared in pigs and rabbits, and serums from noninfected pigs and rabbits, were fractionated with Rivanol and ammonium sulfate. The gamma globulin fractions were conjugated with fluorescein isothiocyanate. Infected PK cells (a transmissible line of swine kidney cells), primary swine kidney cells, primary swine testicle cells, primary swine bone marrow cells, and noninfected cells of the same origins, were exposed to the labeled antibody. Smears prepared from the peripheral blood and lymph nodes of swine acutely sick with hog cholera, were treated the same as cultures. None of the infected cultures nor the smears were suitable for demonstration of specific immunofluorescence since the nonspecific reactions were of the same amount and intensity in both the infected and noninfected cells.

Studies were conducted on a PK swine kidney cell line persistently infected with an agent that immunized against hog cholera virus. The transmissible line of swine kidney cells (PK) was received from a commercial company. Apparent rapid modification of virulent virus used as inoculum and marked fluorescence of noninfected cultures suggested that the PK cells were already carriers of an agent antigenically related to hog cholera virus. The presence of the contaminating modified agent was demonstrated by immunizing susceptible pigs against hog cholera with supernatant fluid from an uninoculated PK cell culture. Confirmation that the cell strain was contaminated prior to its arrival at the Hog Cholera Station was demonstrated by immunizing susceptible pigs with another PK cell culture from the original source, immediately after receipt at the Station. It was not possible to eliminate this immunizing agent from the cell strain by using hog cholera hyperimmune serum in the tissue culture medium.

Immunizing properties of hog cholera vaccines. Investigations were made of the factors influencing the potency and immunizing ability of hog cholera vaccines in individual pigs and groups of pigs. One lot of virus (1840 ml) was made and tested for variant characteristics, but none were found. It had a titration of 2,500,000 minimum lethal doses per milliliter. This virus will be used for exposure of swine in various kinds of experiments and supplying experiment stations doing research on hog cholera.

An experiment was carried out to show the relative susceptibility to hog cholera virus of inbred lines of Hampshire, Duroc, Poland China, Yorkshire Landrace, and Chester-white pigs. Four of 6 Hampshires died; 5 of 6 Durocs died; 1 of 6 Poland-Chinas died; 1 of 5 Yorkshires died; none of the 6 Landrace died, and 1 of 2 Chester-whites died. The Durocs and Hampshires seem to be the most susceptible.

Crystal violet glycerol (CVG) vaccine, Sn. 116, that was made in 1959 and used to vaccinate farm herds in 1960, was re-tested and found to give 78 percent protection. The percent protection had not changed since being tested a year previous. Another vaccine, Sn. 117, consisting of 124,000 ml. was prepared in 5 sub-lots. Some of the sub-lots and the final pool were tested in Florida. All protection tests proved satisfactory. Part of this vaccine, 112,500 ml. was shipped to the Live Oak Station to be used in an experimental eradication trial in Lowndes County, Georgia.

CVG vaccination of 123 herds on 60 farms, composed of 12,088 pigs, was carried out in 1960-61. The immunity of a total of 486 pigs was challenged, 244 head at 1 month, and 242 head at 3 months after vaccination. The total percent protection for the year was 69.22. Last year it was 64.7. Death loss was 13.99 percent or 1.87 percent more than last year, although there was an increase of 13.13 percent in the number of normal pigs and a decrease of 7.78 percent and 7.55 percent in the slight and severe reactors, respectively. Two lots of vaccine serials 115 and 116 were used in this year's vaccination. Two viruses, serials 313 and 316, were used for the challenge of immunity.

Those herds which had little or no protection from single vaccination had 100 percent protecticn when given 2 doses of vaccine 1 month apart. Twenty-five sows, given 2 doses of CVG vaccine 1 month apart, had no reaction to exposure with virulent virus at 12 or 18 months after the last dose of vaccine.

The work on the reactivation of the viruses in modified vaccines was completed and published. Six modified viruses passed serially through pigs, all regained virulence so that they either made the pigs sick or caused death. Three of these viruses usually were transmitted from the injected pigs to the contact control pigs. The transmissibility by contact of the other 3 vaccines was not tested.

Some viruses of lapine origin regained their virulence in 6 passages. Other viruses of lapine origin required 19 passages before they produced sickness in susceptible pigs. A virus of porcine origin caused a severe reaction in pigs on the first passage when no serum was given with the virus. It increased in virulence on subsequent passages.

The addition of 0.15 percent of B-Propiolactone to hog blood that contained cholera virus and incubated at room temperature for 1 hour, killed the virus so that no reaction was produced when injected into susceptible pigs. These pigs developed sufficient immunity to survive a 2 ml. dose of virulent hog cholera virus. However, they were sick from 2 to 5 days. Similar results were obtained by adding 0.16 percent B-Propiolactone to blood containing hog cholera virus and incubating at room temperature for 30 minutes, or by adding 0.32 percent B-Propiolactone to blood containing hog cholera virus and incubating for 30 minutes.

Six accession lots of pigs had varying degrees of protection when 6 pigs from each lot were injected with graded doses of the same serial of vaccine and challenged with the same virus. Some lots had 100 percent protection while other lots had little or no protection.

Another lot of 42 pigs, composed of 6 breeds of pigs, seven in each breed, were all given graded doses of the same vaccine to determine if a genetic factor had any influence on their inability to develop immunity. All of the Poland China pigs died when challenged 21 days after vaccination. All except one of the Landrace died. All of the Chester-Whites survived. Four of the 6 Hampshires survived. Four of the Yorkshires survived, and 4 of the Durocs survived. This seems to indicate that there is a difference in breeds as to their ability to develop immunity.

In 1962, at the National Animal Disease Laboratory, immunizing studies were continued with the vaccination of a farm herd of 450 pigs, composed of three age groups, with crystal violet glycerol vaccine. One month later part of each group was given a second injection of CVG. Six months later representatives from each age group with single and double vaccinations were given hog cholera virus. Older pigs, vaccinated after weaning, were better protected than younger age groups. Double vaccination increased the protection 59 percent in the older group and 29 percent in the younger group. Double vaccination shortened the recovery period from 10 days to 8 days.

White cell counts of these animals showed that the rate and extent of drop in numbers of cells following virus challenge was about the same for single and double vaccinates of all age groups. The return to normal cell count was two days faster in the double vaccinates and the increase in number of cells was greater in the double vaccinates. The rate of recovery to a normal condition is related to the increase in leucocytes. Bacteriological studies of the hogs that died showed no pathogenic organisms present.

One lot of Crystal Violet Glycerol vaccine, consisting of 322 liters was made in 8 sublots. It is being tested for potency.

A total of 606 agar-gel precipitin diagnostic tests for hog cholera were made. Of 414 positive tests, 79 percent were obtained with normal pancreas material and 58 percent were obtained with pancreas from hog cholera infected animals.

The Taylor test for hog cholera, when made on a selected group of 22 virus-only-treated pigs and on vaccinated-and-virus treated hogs gave clear positive tests in 65.5 percent of the animals. The test was doubtful in 31 percent of the cases and 3.4 percent negative.

Bacteriological study of the organs from 630 hog cholera-infected pigs gave isolations of organisms from 114, divided in the following proportions: Escherichia coli 34; Staphylococcus spp. 29; Pseudomonus spp. 14; Streptococcus spp. 13; Proteus spp. 12; Diplococcus spp. 5; Aerobacter spp. 3; Corynebacterium spp. 2; Pasteurella spp. 1, and Escherichia fruendii 1.

Field evaluation of modified live-virus hog cholera vaccines. In 1961, 30,872 pigs from 1,023 herds on 517 farms in Suwannee County, Florida, were vaccinated with modified live-virus vaccines and antiserum. Approximately 85 percent of the swine in the county were vaccinated. Lapine origin vaccine was used on 35 percent of the pigs; porcine origin on 38.4 percent of the pigs, and tissue culture origin vaccine on 26.6 percent of the pigs.

Immunity tests on 661 randomly selected market-age swine from 340 herds showed that 82.5 percent of the pigs were adequately protected. This was an increase over the 78.1 percent protection from the previous year. The increase probably resulted from the use of fresh vaccines in recent months, and it supports the hypothesis that there is an inverse relationship between the age of the vaccine and its protective potency.

There were 13 confirmed hog cholera virus isolations; 7 from non-vaccinated swine and 6 from vaccinated swine. Thus more than 50 percent of the virus isolations came from the 15 percent of the swine that were not vaccinated. All 13 positive cases occurred during the first half of fiscal year 1961. This was a significant decrease from the 24 isolations in 1960. It appears that the high level of vaccination coverage has kept the disease well under control.

An investigation of immunization failure is centered around possible genetic resistance to immunization. Breeding stock was obtained from a herd from which only 10 of 19 animals were adequately protected by vaccination. In

preliminary studies, 2 of 6 first-generation pigs were susceptible to virus challenge given 104 days after vaccination with modified live-virus vaccines. One of 2 second-generation pigs was susceptible when given an immunity challenge 80 days after vaccination.

Titration of 6 different vaccines were carried out to determine minimum protective doses. There was a great variation of potencies among the vaccines tested. Further work is needed on the titration of vaccines as soon as possible after production and at 6-month intervals until the expiration date in order to plot a reliable immunogenicity retrogression curve.

In 1962 in Suwannee County, Florida, 9,307 swine were vaccinated against hog cholera with lapine origin vaccine, 295 of them were challenged, and 260, or 88.1 percent were adequately protected. A total of 10,931 swine were vaccinated with porcine origin vaccine, 321 of them were challenged and 278, or 86.6 percent were adequately protected. A total of 9,622 swine were vaccinated with tissue culture vaccine, 191 of them were challenged and 165, or 86.4 percent were adequately protected. The totals for all types combined were 29,860 swine vaccinated, 807 challenged, and 703, or 87.1 percent adequately protected.

Porcine origin vaccines recovered from a low percentage of adequately protected pigs of 61.6 percent in fiscal year 1960 to a high of 86.6 percent in fiscal year 1962. The average age of porcine origin vaccines (shelf-life) in fiscal year 1960 was 564.4 days (more than 18 months) whereas the age of the same type of vaccine in fiscal year 1962 was only 206.3 days (less than 7 months). The poor showing of porcine origin vaccine in fiscal year 1960 involved 8 serial numbers from 4 manufacturers. The recovery of this vaccine in fiscal year 1962 involved 6 serial numbers from 3 manufacturers.

The efficacy of all porcine origin vaccines during the past 6 years shows approximately the same pattern as described above. The average percentage of adequately protected pigs was 88.5 when the vaccines were less than 1 year old. When the vaccines were between 1 year and 18 months of age, the percentage of adequately protected pigs dropped to 80.3, and when the vaccines were more than 18 months of age, this figure was 57.9 percent. Lapine origin vaccines and tissue culture vaccines showed similar correlative declines but they were not as marked and not as early. With lapine origin vaccines, 88.5 percent of pigs were adequately protected if the vaccines were less than 18 months of age, whereas, this figure was only 80.9 percent when the vaccines were more than 18 months of age. (The minimum acceptable percentage of adequately protected pigs is 80.0 percent). With tissue culture vaccines, 91.9 percent of pigs were adequately protected when the vaccines were less than 18 months of age, and 87.8 percent were adequately protected when the vaccines were more than 18 months of age.

During fiscal year 1962, a field trial study was started in Lowndes County, Georgia, in cooperation with the Animal Disease Eradication Division of ARS and the State of Georgia, under the terms of a Memorandum of Understanding. This arrangement was similar to the one in Suwannee County, Florida, to determine the efficacy of inactivated and killed vaccines. Formal agreements were entered into with 703 swine owners, which is about 97 percent of the total swine owners in the county.

During fiscal year 1962, in Lowndes County, Georgia, a total of 23,899 swine were vaccinated with Boynton's Tissue Vaccine (BTV), and experimental Crystal Violet vaccine (ECVG) and a commercial CVG vaccine (CCVG). A total of 5,289 swine were vaccinated against hog cholera with BTV, 9 of them were challenged and all 9 were adequately protected. A total of 9,963 swine were vaccinated with ECVG vaccine, 50 of them were challenged and 49 were adequately protected. A total of 8,647 swine were vaccinated with CCVG vaccine, 10 of them were challenged and all 10 were adequately protected. Additional swine receiving each type of vaccine will be challenged when they reach market age.

Three positive cases of hog cholera in Suwannee County, Florida, were disclosed in non-vaccinated, farm-raised swine on 2 farms and in vaccinated, farm-raised swine on 1 farm. In Lowndes County, Georgia, hog cholera was confirmed in purchased, non-vaccinated swine on 1 farm.

In other tests, in Suwannee County, Florida, it was shown that 2 doses of inactivated vaccine administered 30 days apart, imparts almost 100 percent immunity, even if serum is administered simultaneously with the first dose.

Development of a rapid diagnostic test for hog cholera. In January 1962, this work, carried out at the University of Illinois, under a cooperative agreement was initiated. Preliminary investigation indicates that a hem-agglutination test has promise as a rapid diagnostic test for hog cholera.

The relationship of hog cholera to bovine mucosal disease. In 1962 work was carried out under a cooperative agreement on the mucosal disease-virus diarrhea complex of cattle at the College of Veterinary Medicine, Iowa State University. The experimental results indicate that the Sanders mucosal disease agent does not give uniform protection to swine against virulent hog cholera virus. The results definitely indicate that the Sanders Mucosal Disease Agents affords protection in some of the swine against virulent hog cholera virus.

One experiment, consisting of 8 pigs, has been conducted in this area of study so far. The pigs were divided into 4 lots of 2 each. Two pigs were inoculated intravenously; 2 subcutaneously and 2 intramuscularly with Sanders Mucosal Disease Agent. Two pigs remained as controls. The only evidence of response to the inoculation was a drop of about 50 percent in the total leukocytes between the 4th and 6th days postinoculation.

Nineteen days later all 8 pigs were inoculated subcutaneously with virulent hog cholera virus. The 2 control pigs developed clinical evidence of hog cholera 3 days postinoculation and were destroyed in extremis on the 9th and 11th days postinoculation. Typical lesions of hog cholera were observed at necropsy.

One of the pigs which had been inoculated intravenously with Sanders agent developed clinical symptoms of hog cholera on the 2nd day post-challenge and was destroyed in extremis 15 days later. Lesions of hog cholera were evident at necropsy. The second pig to receive the intravenous inoculation of Sanders Agent displayed a mild pyrexia, depression, and weakness in the rear quarters between the 4th and 14th days post-challenge. After this period the pig showed no evidence of illness and apparently returned to normal health.

Of the two pigs that received the Sanders Agent intramuscularly, one showed little evidence of infection following the hog cholera challenge, the other pig, however, was quite ill between the 4th and 12th days post-challenge. During this period the temperature varied from normal to 106°F. The animal was very weak, depressed and ate very sparingly. After this period the pig rapidly returned to normal.

One of the two pigs that received the subcutaneous inoculation of Sanders Agent developed typical symptoms of hog cholera on the 3rd day post-challenge and was destroyed in extremis on the 9th day post-challenge. Lesions of hog cholera were evident at necropsy. The other pig to receive the Sanders inoculation subcutaneously developed symptoms 6 days post-challenge and was ill for 7 days. During this time the pig was markedly depressed, off feed and showed a variable temperature elevation. Following this period the pig rapidly returned to normal.

B. Atrophic Rhinitis.

In 1961, at the Animal Disease Station, Beltsville, Maryland, studies were carried out on the causative agent or agents, mode of spread, diagnosis, and control of atrophic rhinitis. Progress in the atrophic rhinitis (AR) project is developing methods of inoculation and storage of inoculum pools during this period was satisfactory, and data obtained further substantiated findings of previous years. The contributions were (1) frozen inoculum will remain viable for at least 8 months, whereas previous tests of frozen inoculum showed viability for only 4 months; (2) the material can be diluted out at least 40 times and still remain infective. Previous tests were made only with dilutions of 1:3.5; (3) the transmissible nature of AR infected nasal turbinate mucus membrane tissue was clarified by experiments testing the noninfected materials and conditions associated with positive transmission. Air alone, saline alone, or normal nasal turbinate mucus membrane tissue did not produce atrophy of the turbinates when instilled into the nasal cavity of susceptible pigs by means of the DeVilbiss atomizer with an electric pump at 13 pounds pressure; (4) the atrophic rhinitis susceptibility of pigs 14 to 25 days of age was similar to previous years experimental results with pigs under 14 days of age, and a few swine over 2 months of age, when tested, were susceptible to atrophic rhinitis exposure; (5) streptomycin or a combination including streptomycin, will inhibit atrophy-producing ability when mixed with the inoculum, whereas other antibiotics tested (penicillin, polymyxin or bacitracin) will not; (6) data obtained shows lyophilization seems to be the poorest method of storage of the inoculum; (7) the use of the rhinoscope may be of some value for diagnosing AR in a herd, but is not efficient enough for the critical work required of it for research.

In 1962 work was initiated at the National Animal Disease Laboratory, Ames, Iowa. Work during the past year was associated with the hiring and training of new project personnel, testing the established AR-free swine herd for susceptibility to the disease, developing and testing new animal cage isolation equipment and testing the deep freeze stored AR material from Beltsville for viability and transmissibility to susceptible pigs. The development of closed air and sewage systems in a plexiglass Horsfal-Bauer type isolation cage, 24"x24"x36" used in 3 experiments maintained satisfactory isolation between AR infected and normal control pigs up to 100 pounds live weight for approximately 60 days. The 20 AR-free, purebred Yorkshire sows moved from Beltsville, Maryland, have provided a satisfactory source of susceptible pigs. The atrophic rhinitis frozen agent harvested and shipped from Beltsville was viable and transmissible to pigs in a 1:3.5 dilution after 16 months storage. It was transmissible to pigs in a 1:56 titration dilution after 18½ months storage. However, the pig passage of epithelial tissue harvested from the NADL experimental pigs was negative. Swine age susceptibility tests are in progress.

C. Transmissible Gastroenteritis

In 1961 preliminary work at the National Animal Disease Laboratory, Ames, Iowa, was directed toward producing disease-free swine for use in controlled experiments on transmissible gastroenteritis (TGE).

In 1962 at NADL, two lines of cells, one from fetal swine testis and one from fetal swine kidney, were developed in this laboratory for use in research on TGE. Only the swine testis cells have been used thus far. Characteristically these cells grow very slowly and require 5 percent CO_2 in the atmosphere for optimum growth. The cells have been passed only 16 passages in the 8 months since they were started. Eight passages were made in the first 3 months and 8 passages have been made in the last 5 months.

Investigations utilizing two isolates of transmissible gastroenteritis have been undertaken. One isolate obtained from a natural field outbreak in Central Iowa, and a second isolate received from the Purdue University, were used. It is the original Indiana isolate of TGE. Both isolates were pathogenic for very young specific-pathogen-free (SPF) pigs.

The Iowa isolate was inoculated into the swine testis cells at the 8th passage of the cells. The cytoplasm of many of the cells became granular and there was a marked acceleration growth pattern of the passaged cells as compared with noninoculated control cell line. The persistently infected cells have been passed 20 times since they were infected 5 months previously. They grow well without CO_2 atmosphere and in Earle's balanced salt solution and 10 percent pig serum.

Positive evidence of viral invasion of the cells was obtained by feeding 2 six-day-old SPF pigs infected cells after they had been passed 15 times. The infected pigs showed no overt signs of disease, but when they were challenged, along with the control pig, with the original homologous virus isolate, only the control pig developed clinical evidence of TGE.

The Purdue isolate was passed 3 times in an established line of pig kidney cells obtained from another laboratory. There was no evidence of infection or multiplication of the virus in these tissue culture cells. Positive evidence of viral invasion of the cells was indicated when the 3rd passage of the tissue culture passed virus was fed to each of two 6-day-old SPF pigs. The virus appeared to be partially attenuated as only one pig developed signs of TGE and died. This pig had lesions suggestive of TGE. The second pig showed no signs of disease. When the remaining pig and a control pig were challenged with pig passaged homologous virus only the control pig developed clinical evidence of TGE.

In 1961, under a cooperative agreement with the University of California, at Davis, studies were conducted with 4 of 6 viral agents isolated from the intestine of young pigs suffering from an acute scouring in the field. Investigations were conducted to determine the optimum conditions under which the 4 agents could be propagated and demonstrated by tissue culture methods.

A neutralization test, using the tissue culture system, showed an antigenic relationship among the viruses obtained from pigs with scours which were serologically unrelated to a viral agent obtained from an apparently normal hog.

Investigations on the physical properties of the viruses were carried out. Exposure at 56 C for 40 minutes inactivated 90 percent of the virus but infectious material was still present after 2 hours; after 5 hours no infectious virus remained. The viruses were resistant to 20 percent ether, 1 percent trypsin, and were viable after 300 days at -20 C. None of the viruses hemagglutinated red blood cells of chickens, guinea pigs, sheep, or swine.

Preliminary trials with one of the agents obtained from the scouring pig resulted in signs of leg weakness, paralysis and definite histopathological lesions in the brain stem and spinal cord, when inoculated into 24-hour-old specific pathogen-free pigs.

In 1962, at the University of California, physical, serological and pathological studies were performed with four strains of porcine enteroviruses designated as E1, E2, E3, and E4. The particle size of these agents was estimated to be 33 to 46 mu in diameter by gradocol membrane filtration. The relationship between these four strains was studied by agar gel diffusion test, and further work is now in progress.

The porcine enterovirus (PE-1 strain) isolated by the Canadians differed from our four strains as determined by the tissue culture plaque neutralization test. Pathogenesis of the four strains was studied by oral inoculation into SPF pigs. In most cases, the pigs inoculated with E1, E2, or E3 strain showed signs of gastro-intestinal enteritis, and the virus was isolated from the lower intestinal tract. One of five pigs inoculated orally with the E1 strain showed paralysis in the hind legs and poliomyelitis lesions were demonstrated in the spinal cord by histopathological examination.

In 1961, under a cooperative agreement with Purdue University, Lafayette, Indiana, duration of immunity to transmissible gastroenteritis was tested by immunizing 6 gilts by oral exposure to virulent virus during pregnancy. In the first farrowing their pigs had morbidity of 19 percent and mortality of 12 percent. Fifteen months after the first farrowing, the sows farrowed again and on challenge of the pigs at 3 days of age, the pigs showed morbidity of 11 percent and mortality of 8 percent. Three of 4 sows conferred solid immunity to their pigs. To test the duration of the carrier state, fecal samples were taken from 35 pigs at weekly intervals for 11 weeks and tested for the presence of TGE virus. The 35 pigs were left with the sows until weaned at 6 to 7 weeks. Of 375 fecal samples, 188 were tested by inoculating individual pigs in isolation. TGE virus was demonstrated in only 10 of 35 pigs in the first week, from 2 pigs in the second week, and none in the weeks thereafter. The results are at variance with the only previous report on the subject in which pigs shed TGE virus for as long as 8 weeks after exposure.

In 1962, at Purdue University, two phases of immunity to TGE were studied. In continuing the work on duration of immunity, it was found that gilts infected as suckling pigs between 17 and 25 days of age did not retain sufficient immunity to protect their litters from our standard challenge after one year. There was some prolongation of incubation period and some diminution in death loss over controls.

Antibodies absorbed from colostrum through the gut of the newborn animal are considered to be the important mechanism of transfer of immunity from dam to young in most farm species including swine. Experiments were done in which pigs were transferred from immune sows to non-immune sows and vice versa. It was shown that immunity against TGE transferred from sow to pig is dependent upon a continuous supply of "immune" milk. Intraperitoneally inoculated anti-serum failed to protect pigs while the same anti-serum protected when fed in the milk indicating that the important site of action is probably in the lumen or walls of the alimentary tract.

Persistence of virus shedding was not studied extensively because this work requires large numbers of pigs which were in temporarily short supply. However, it was found again that three infected pigs did not shed virus after one week. Four-day-old pigs shed as much as 10,000 infectious doses of virus per gram of feces as early as 24 hours after infection.

New approaches to adapt TGE virus to tissue cultures were made this year involving kidney monolayer cultures, organotypic cultures of various visceral organs including various parts of the gut, and a modified organ culture technique patterned after Warren's technique for growing polio-virus in monkey gut. While none of these was completely successful, the method of growing explants of intestine on millipore filters is being developed and in the best cultures would at least maintain TGE virus for three to four days.

D. Swine Erysipelas

In 1961, at the Animal Disease Station, Beltsville, Maryland, fifty pigs, farrowed by vaccinated swine-erysipelas-immune dams, were divided into 5 groups of 10 pigs each after weaning. They were vaccinated with anti-swine erysipelas serum Lot G, and commercial desiccated virulent Ery. rhusiopathiae at different mean ages after birth (59.7 to 115.7 days). All were exposed to infection 94 days after vaccination, including 14 pen-contact controls, by the percutaneous method of exposure. There was a trend of improvement in the immunizing response to vaccination as the age when vaccinated was increased. All pen contacts were susceptible. The experimental results indicated that pigs farrowed by immune dams should not be vaccinated with virulent culture and serum until after they are 3 months old.

Biochemical investigations were carried out in cooperation with the Department of Biochemistry, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey. Buffer extracts of Ery. rhusiopathiae were resolved into 5 antigens that were distinguishable on the basis of (1) chromatographic properties, (2) heat stability, and (3) reaction of non-identity in agar diffusion studies. The antigens were identified as homologous, isologous and heterologous with respect to known serotypes.

In 1962, at the National Animal Disease Laboratory, Ames, Iowa, work was carried out as follows: 1) No gross evidence of arthritis was observed in rabbits after sensitization to Erysipelothrix rhusiopathiae in combination with Fruend's adjuvant, when injected intradermally in the interdigital space and side. 2) Acetone powder was prepared from 147 liters of broth culture for the immunological and chemical characterization of the antigen components of Ery. rhusiopathiae. 3) Peak A, the type-specific antigen derived from acetone powder, is identical with the acid extracts of the cells that formed the basis for the original classification of strains of E. rhusiopathiae.

Biochemical investigations were continued cooperatively with the Department of Biochemistry, Seton Hall College of Medicine and Dentistry, Jersey City, New Jersey. Ion-exchange chromatography of neutral extracts of acetone powders of Ery. rhusiopathiae strain S-192 on DEAE-cellulose columns resulted in the isolation of 5 serologically distinct antigens when assayed by the agar double diffusion technique. Peak A, the "breakthrough" peak from the column precipitated only with homologous anti S-192 serum and the antiserum to a closely related strain NF4.

During the period covered by this report, purification of the serologically active material in Peak A by chromatography, acetone fractionation, and exhaustive dialysis yielded a preparation which had all of the serological activity of the original Peak A.

Studies on the chemical composition of this type-specific antigen have indicated that it is a mucopeptide most probably derived from the cell wall of the organism. It has been demonstrated that the antigen isolated in this manner from acetone powders of the organism is identical both chemically and serologically with the antigen present in acid extracts of the organism. It was acid extracts of cells of Erysipelothrix which were used originally to type the various strains of the organism.

E. Brucellosis.

Under a cooperative agreement on bovine brucellosis, reported in Area 1, with the College of Veterinary Medicine of the University of Minnesota, studies on brucellosis in swine were undertaken in April of 1962.

Sows and boars which react to the tube seroagglutination test for brucellosis at the time of slaughter are being traced to the farm of origin. These selected herds are followed and evaluated using epidemiologic and serologic methods developed for the detection of frank and inapparent infections of brucellosis in cattle. These studies include attempts to isolate the organism from all animals which leave the herd for slaughter using culture media, guinea pig inoculation, and fluorescent antibody micropsy.

Blood has been collected from 363 sows which have been identified prior to slaughter in order that all of the animals from which blood was collected may be traced to the herd of origin. These sows are selected with the cooperation of a packing plant, and the herds of origin are located in southern

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Minnesota and northern Iowa. Initial serological studies include the plate sero-agglutination test, tube sero-agglutination test, acid plate antigen test, heat inactivation test, rivanol precipitation plate sero-agglutination test and in some cases the 56°F heat inactivation test. In the herd studies, the 56° HIT and MCE tests will be included on all animals. Preliminary results indicate that the number of reactors to the plate and tube agglutination test at dilutions of 1:100 and greater are quite low; however, approximately 10% of the animals show plate or tube reactions at 1:25 or 1:50 dilutions. The results of the supplemental tests are now being evaluated. Trace-backs are in progress to locate the herds of origin of those animals which reacted.

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AREA NO. 3 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF SHEEP AND GOATS

Problem. There are at least 18 infectious diseases of sheep and goats in the United States that cause an estimated annual loss of 15 million dollars. Non-infectious diseases are estimated to cause an additional 3 million dollar loss annually. The cause of some of these diseases is known; others have more than one causative agent contributing to produce the effects seen in field cases. Environmental, genetic, and unknown factors appear to play a part in some diseases. The natural reservoirs of the known infectious agents have not been fully determined. Fundamental information on methods of transmission and means of prevention are needed for many of these diseases. Vaccines and other immunizing products are available for some diseases of sheep but not for others. Some of these products might be improved. Prevention, control, or eradication of disease is necessary for economic and efficient sheep and goat raising. Due to lack of accurate, rapid diagnostic techniques, infectious diseases often get a substantial start in a band or flock before they are recognized, partly because they are easily confused with non-infectious diseases.

USDA PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of sheep and goats. Research is being conducted on the diseases at the following designated locations.

The Federal scientific effort devoted to research in this area totals 6.6 professional man-years. This effort is applied as follows:

Bluetongue, 2.0 at the Denver Animal Research Laboratory, Denver, Colorado.

Contagious Ecthyma, 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Foot Rot, 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Scrapie, 0.2 at the Agricultural Research Council Field Station, Compton, Berkshire, England, and the Moredun Institute, Edinburgh, Scotland, through two grants of P.L. 480 funds, equivalent to $300,165. The work is coordinated through the European Mission for Research on Animal Diseases, Amsterdam, Holland.

Vibriosis, 0.3 under cooperative agreements with the Colorado, Montana, and Utah Agricultural Experiment Stations.

Viral Ulcerative Dermatosis, 0.1 through a cooperative agreement with the Colorado Agricultural Experiment Station.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 11.3 professional man-years divided among subheadings as follows: (a) Vibriosis 5.6, (b) Scrapie 0.2, (c) Bluetongue 0.5, (d) Other diseases (pneumonia, mastitis, caseous lymphadenitis, listeriosis, etc.) 5.0. Regional research project W-27, Vibriosis in sheep, coordinates investigations on vibriosis of sheep between six western states and the USDA. Studies are being made at Indiana on scrapie. California, Texas, and Washington are working on bluetongue. California and Ohio are conducting research on the cause and prevention of pneumonia in lambs. Montana is studying the causes of mastitis in ewes and developing practical methods for control, and has work under way to identify the cause of balanoposthitis in rams and to develop procedures suitable for its control. Caseous lymphadenitis-its cause and prevention- and improved treatments for pregnancy disease of sheep are research objectives at Missouri. Nebraska and North Dakota are elucidating factors which contribute to outbreaks of listeriosis in sheep. South Dakota and Wyoming are working on the cause and prevention of urinary calculi in sheep. California is studying the cause and prevention of encephalomalacia in lambs and the significance of eperythrozoonosis of sheep.

Industry and other organizations are engaged in the preparation of marketable biologic and pharmaceutical products. They conduct experimentation on vaccines and the formulation of chemical compounds and other medicinal substances for prevention and treatment of diseases of sheep and goats. These companies generally will utilize their own facilities. Information gained in their research generally is confidential in nature as are expenditures for research and development. It is estimated that 20 p.m.y. are devoted to this work by industry and other organizations.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Bluetongue

In 1961, at the ADP Denver Laboratory, sheep blood samples were tested, representing suspected bluetongue (BT) outbreaks in 11 flocks from 4 States in which 6 flocks from 3 States were found to be infected. Bovine blood samples of 38 herds from 6 States were tested for BT virus, with isolation from 3 calves in a Utah herd, and 1 calf in an Idaho herd. These virus isolates were typed in sheep and found to be of the same immunogenic type as our standard BT virus.

Two of 3 mature goats, inoculated with known virulent BT virus, showed no clinical symptoms or febrile response to the disease. The third goat, although showing no clinical symptoms, did show a febrile response from post-inoculation days 9 to 12. All three goats withstood challenge with the homologous virus.

Sheep inoculated with 17th, 30th, and 40th serial passage tissue culture virus developed an immunity to the disease as evidenced by challenge with known virulent BT virus and tissue culture serum neutralization tests. Sheep inoculated with whole blood from sheep receiving 17th, 30th, and 40th passage tissue culture virus exhibited no visible clinical symptoms but did withstand challenge from known virulent BT virus. Further passages of the tissue culture virus onto tissue culture cells and subsequent testing in sheep will be continued.

Attempts to isolate the virus from whole blood of 13 reacting sheep were successful in 2 cases. Four serial blind passages in tissue culture were made on the blood from the other 11 animals, with negative results.

Serum neutralization tests, using a tissue culture virus or concentrated chicken embryo virus, were conducted on serum from 186 sheep, including 56 animals used in the Culicoides variipennis (flies) transmission studies. Normal serum and serum from sheep exposed to the BT virus but not infected, displayed no specific BT antibodies, whereas the serum from those animals exposed to the BT virus and followed by a resultant infection to bluetongue consistently showed a specific log index of 3.5.

Studies on the transmission of bluetongue in sheep by insect vectors is a cooperative project with the Entomology Research Division, Kerrville, Texas, with emphasis directed toward Culicoides variipennis.

Eighteen flies (colony raised) that fed on a bluetongue infected sheep transmitted the virus 15 days later when they fed on a susceptible sheep. However, 19, 10, and 2 flies which had fed on a BT infected sheep did not transmit the disease when fed 13 days later on susceptible sheep. However, the 2 aforementioned flies, which were immediately processed after feeding on the susceptible sheep, transmitted the virus when injected into a susceptible sheep. The aforementioned 19 flies were immediately processed and subsequently passaged on tissue culture cells with isolation of the BT virus as evidenced by cytopathogenic effect (CPE), serum neutralization tests, and inoculation of sheep.

Sixty-one flies that fed on a BT-infected sheep, and then 10 days later fed on a susceptible sheep, did not transmit the virus. These flies were immediately processed and injected into a susceptible sheep with transmission of the virus.

Twenty-seven flies that fed on a BT-infected sheep and then fed on a susceptible sheep 15 days later, did not transmit the virus. Thirty-seven and 122 flies that fed on a BT-infected sheep transmitted the BT virus to their respective susceptible sheep upon taking their second blood meal 10 days later.

Thirty-eight, 1, 10, 36, and 20 flies that fed on a BT-infected sheep took their second blood meals 13 days later (except for the 38 flies which fed 10 days later) on their respective susceptible sheep with no transmission of the virus.

Six lots of 43 flies each, after feeding on a BT-infected sheep, did not transmit the virus after taking their respective second blood meals 8, 9, 10, 13, 15, and 20 days later on susceptible sheep. Four lots of 1 fly each did not transmit the virus when fed on susceptible sheep 16, 19, 20, and 21 days later, respectively, after taking their first blood meal on a BT-infected sheep.

Six and 2 flies that fed on a BT-infected sheep, took their respective second blood meals on susceptible sheep 16 and 20 days later with negative transmission of the virus. Fifty-three, 107, and 100 flies, after feeding on a BT-infected sheep, failed to transmit the disease 10 days later when they fed on their respective susceptible sheep. One and 47 flies failed to transmit the virus when fed on their respective susceptible sheep 13 days after having fed on a BT-infected sheep. One hundred six flies, which had fed on a BT-infected sheep, did not transmit the virus to a susceptible sheep when fed 7 days later. Three and 6 flies, when fed on the same susceptible sheep 12 and 13 days respectively after having first fed on a BT-infected sheep, did not transmit the virus.

Approximately 2,500 flies which had fed on BT-infected sheep but not exposed to susceptible sheep for a second blood meal, were incubated in varying lots for integrated periods of time ranging from day zero through 14, at which times they were stored at -60 F.

All flies, except those processed at the time for virus isolation, used in the direct BT transmission studies, including those exposed to a susceptible sheep but not taking a blood meal, were stored at -60 F.

Sixty-eight flies which had fed on a BT-infected sheep, were found to be infective to a susceptible sheep after having been incubated for 10 days and then stored in a dead state at room temperature for 10 days.

Seventeen flies, held for 18 days after feeding on a BT-infected sheep, were placed in an airtight vial containing a minimal amount of Mycostatin and then stored for 6 days at room temperature, were not found to be infective to a susceptible sheep.

Five lots of flies incubated 10, 10, 13, 13, and 15 days, respectively, following a blood meal on BT-infected sheep, were stored for over 5 months at -60 F., at which time the flies were processed for attempted viral isolations in tissue culture and sheep. Viral isolation was successful in both sheep and tissue culture in the single instance of 1 lot of flies which had been incubated 10 days prior to storage. Virus isolation in sheep from 1 lot of flies incubated 13 days was successful, whereas the remaining virus isolation attempts were negative.

Four viral isolates, one of which is a suspected field variant, are being typed in sheep and by tissue culture methods. Results are inconclusive, so further testing is in progress. (Denver, Colorado)

The technique of agar diffusion was applied to a study of bluetongue virus antigens prepared from infected mouse brains, chicken embryos and cell culture fluid. Antigens from these different sources, contributing to precipitate formation, were virus-specific, noninfective and serologically indistinguishable one from another in the systems tested. The onset and production of circulating ovine anti-bluetongue virus precipitin was correlated to corresponding data for homotypic virus infectivity neutralizing antibody. The onset of precipitin formation was detected about the same time as for neutralizing antibody, but the precipitin persisted longer. An anamnestic response was observed for neutralizing antibody but not for precipitin. An antigen-antibody system, containing one component in weak concentration, precipitated if adjacent to a positive system but not if placed by itself. This observation, termed the "recruiting effect," can influence quantitation of precipitin. (Pullman, Washington)

Female mice were immunized with bluetongue virus just prior to or during pregnancy. Only lacteal transfer of neutralizing antibody to bluetongue virus was demonstrated from immune mothers to their offspring. However, the existence of in utero transfer at a very low level was not excluded.

The passive immunity of the offspring gradually increased during the first 12 days after birth. A marked protection was noticed also in 24-day old mice indicating that mice older than 2 weeks absorbed antibody. After weaning, the passive immunity was lost at a rate that agreed with the reported half-life for mouse antibody of 2.5 to 3 days.

No detectable passive immunity was obtained by offspring of females fed virus-infected material. The use of this finding as a possible diagnostic tool for strain differentiation was discussed. (Pullman, Washington)

In 1962 the Denver Animal Disease and Parasite Research Laboratory was remodeled and equipped for investigations on bluetongue (BT) virus disease of sheep and other animals.

The progress in cell cultures includes the colonization and testing of various ovine and bovine organ cell lines. This research is preliminary to the development of a satisfactory virus-serum neutralization test. It will also furnish an adequate biological medium for basic viral investigations utilizing fluorescent antibody techniques. Homogeneous cell cultures colonized from susceptible body organs will furnish the virus laboratory with a more nearly defined living experimental system. Plaque assay, cytopathogenic studies, serological tests, and virus titrations depend on homogeneous cell lines that will give reproducible results.

The virus has been adapted to embryonating chicken eggs and further studies are being conducted to determine highest titers and maximal yield of viable material from homogeneous egg embryos.

The study of the pathogenesis of bluetongue disease of sheep indicates that: 1. The intradermal route of inoculation is the most effective method of producing bluetongue infection in sheep. 2. The oral route of administration

will not cause a susceptible animal to develop the disease, but may cause sensitization and cause more severe signs and symptoms of disease when later challenged via the intradermal route. 3. Sheep keds (Melophagus ovinus) are capable of transmitting bluetongue disease to sheep. Sheep ked transmission of bluetongue diagnosed by typical signs and symptoms occurred in 16 out of 28 sheep in the insect vector transmission experiments.

The study of field isolates still indicates that there is only one strain of bluetongue virus in the United States. One isolate from California, BT 216, still may be proved to have some slight variation in its antigenicity when compared to California BT 8 which is presently incorporated in the commercial vaccine.

A study of field isolates from cattle indicates that at least three negative subpassages in sheep must be obtained before a negative result should be reported.

The Animal Disease and Parasite and the Entomology Divisions of the Agricultural Research Service have instigated a full time cooperative research project at the Denver ADP Laboratory to further study the problem of the possible transmission of virus diseases to domestic animals by various insects.

B. Scrapie

Scrapie, a generally fatal disease of sheep, was first diagnosed in this country several years ago, but is not considered to be firmly established. An eradication program is in progress.

It is apparent that the two chief factors involved in the disease are a transmissible agent that has not been characterized in detail, and genetic constitution which probably determines susceptibility. Additional information about the disease is needed to improve eradication procedures. Study of the disease has been continued by an ADP animal pathologist in cooperation with the Agricultural Research Council Field Station at Compton, England. In this study of scrapie in experimentally infected goats, it has been determined that the microscopic lesions of the disease are manifest only in the nervous system. In contrast with sheep, all goats have been susceptible regardless of method of inoculation. The disease is characterized by degeneration of nerve cells, especially in the thalamus of the brain. The research under way at two locations in Great Britain is aimed toward characterization of the transmissible agent and clarification of the apparent genetic influence on susceptibility. In Scotland, evidence has been accumulated to show that scrapie can be transmitted to goats by contact with either infected sheep or goats. Confirmation was based upon clinical signs of the disease and laboratory procedures. Four goat kids developed scrapie within 12 months subsequent to intracerebral injection of goat brain material.

An ether-extracted scrapie sheep brain preparation, passed through calcium phosphate and injected into sheep, is believed to be the most "pure" preparation of scrapie material yet shown to be active. This finding makes the work of searching for the causative agent(s) of scrapie more hopeful.

In England a good biochemical approach is being made to isolate the causative agent of scrapie from sheep, goat, and mouse tissue. The injection of goat brain material into several mice induced within 7 to 14 months the onset of signs resembling those of scrapie. The condition has been transmitted from mouse to mouse by inoculation. The infection rate in mice was 100 percent. There is evidence of the adaptation of the agent in mice. These studies could ultimately establish the mouse as an important and economical tool in scrapie research. The recent contact exposure studies with goats strengthen the theory of the contagious nature of the disease under certain conditions.

C. Vibriosis

In 1961, in work in cooperation with the Colorado Agricultural Experiment Station, 250 ewes, grouped at random into 10 isolated lots of 25 animals each, were used to determine: a) minimum concentration of killed vibrio fetus cells to immunize against vibriosis; b) efficacy of mineral oil and alum adjuvant V. fetus vaccines in eliciting a high and lasting immunity. A single adjuvant vaccine contained 0.5 mg., 1.0 mg., or 2.0 mg. V. fetus cells per ml., and was administered in one 5 ml. subcutaneous injection to ewes of a single lot, prior to breeding. Challenge to immunity during advanced gestation was by oral inoculation with virulent V. fetus culture.

No abortions occurred in 63 ewes vaccinated with mineral oil adjuvant vaccine. Three of 69 (4%) ewes aborted after receiving alum adjuvant vaccine. An additional 70 ewes, vaccinated with concentrated cells without adjuvant, resulted in 10 abortions (14%), compared to 22 abortions (88%) occurring in 25 non-vaccinated, orally challenged control ewes.

In 1962 immunization studies on vibrionic abortion in sheep were conducted to determine the protection afforded sheep vaccinated with vibrio fetus serotype I killed vaccine, when challenged with V. fetus serotype V live culture, and vice versa. Primigravid ewes were selected at random to form lots, isolated from each other. Ewes of designated lots, prior to breeding, were vaccinated with a single dose of one V. fetus serotype bacterin. Challenge to immunity during advanced gestation was via the oral route with a measured dosage of the heterologous V. fetus serotype culture.

Ewes vaccinated with one V. fetus serotype bacterin were not protected against vibrionic abortion when their immunity was challenged with the heterologous V. fetus serotype culture.

Current studies, and previous investigations reported by this station, indicate that ewes vaccinated with V. fetus serotype I, or V. fetus serotype V bacterin were immune when their immunity was challenged with a measured dosage of the homologous V. fetus serotype culture. These findings indicate strain specificity immunization against vibrionic abortion caused by one V. fetus serotype.

In 1961, in cooperation with the Montana Agricultural Experiment Station, studies on the reservoir of infection, fecal and vaginal cultures made from 45 ewes 7 months after exposure to V. fetus by either artificial or natural means, failed to reveal evidence of infection. The flock lambed normally the following spring.

A study was made of the pathogenicity of V. fetus which failed to show that vibrios isolated from the ovine gall bladder have marked pathogenicity for the pregnant ewe. However, under the conditions of challenge employed, there was no appreciable difference between vibrios isolated from the gall bladder and a strain of V. fetus isolated from an aborted fetus. Information concerning the loss of pathogenicity by V. fetus cultures would be of value. It is difficult to compare pathogenicity of strains when many of the factors involved are not understood.

Vibrios resembling V. fetus in morphology were isolated from the feces of ewes associated with a vibriosis outbreak and from the feces of virgin ewes without history of contact with V. fetus. V. fetus has not been isolated from the feces of naturally infected ewes at the laboratory of the Montana Agricultural Experiment Station, but was recovered from the feces of artificially inoculated ewes 24 hours after inoculation. Subsequent cultures were negative.

In the evaluation of commercially prepared V. fetus vaccine, the Montana laboratory participated in the 1960-61 field trial of V. fetus bacterin prepared by a commercial laboratory. Seven flocks of sheep, totaling 16,520 breeding ewes, were selected for the trial. The number of ewes actually involved in the controlled experiment was 6,223, of which 3,513 were yearlings and 2,730 were older ewes. The total number of vaccinated ewes was 3,067, and the number of control was 3,156. Lambs were obtained and autopsied from all of the ranches experiencing losses. Vibriosis was not diagnosed on any of the ranches, but ovine virus abortion was diagnosed on 4 ranches. The sporadic nature of ovine vibriosis was again affirmed. It would also appear that the incidence of ovine virus abortion in Montana is somewhat greater than previously thought.

In 1962, at the Montana laboratory, seven isolants from ovine bile were tested for pathogenicity in pregnant ewes by rumen injection in late pregnancy. Six of the isolants produced abortions as did a fetal strain of V. fetus. This finding, in conjunction with the results obtained from previous serologic and physiologic studies leads to the conclusion that many Vibrio cultures isolated from naturally infected gallbladders are actually V. fetus.

Vibrio resembling V. fetus were isolated from two of twelve ovine gallbladders cultured from a ranch which had an abortion outbreak due to V. fetus of rare serotype three years previously. Antigens made from the bile isolants were not agglutinated by antiserums of common serotypes. Serologic comparison between the fetal and bile isolants has not yet been made.

Routine semen cultures which were made from 15 supposedly normal rams resulted in the isolation of V. bubulus from 5 rams and of the ram epididymitis organism from one. This is the first evidence that Montana sheep are infected with the ram epididymitis organism.

Forty-six Vibrio isolants of diverse origin were tested for growth in a medium containing 1 percent glycine. The only group which consistently failed to grow in the presence of glycine was composed of V. fetus cultures of bovine origin. The test is apparently of value in distinguishing between isolants of bovine and ovine origin. Isolants from ovine bile which do not show fair to good growth in the presence of glycine, may not be V. fetus.

Colony studies conducted on Vibrio isolated from ovine bile revealed that many such isolants have colonies which appear to be identical with those observed in ovine fetal strains of V. fetus.

An outbreak of vibriosis, due to serotype I, took place on the Paugh ranch in the spring of 1962. In the fall of 1960,174 Fulton ewes on this ranch were vaccinated with Baldwin V. fetus vaccine (serotype I). There was no evidence of vibriosis in either the vaccinated ewes or the controls in the spring of 1961. In the spring of 1962, 35 Fulton ewes aborted. Ten of these ewes had been vaccinated in the fall of 1960. It would appear that protection the second year following vaccination is not very good; a single additional vaccination in the fall of 1961 might have conferred adequate immunity. Abortions occurred in 7 breeding lots although the rams were of diverse origin. It is extremely unlikely that the rams were the source of the outbreak.

Lambs were obtained for culture from one other ranch (Windsor Livestock) which was on the vaccine experiment in 1960-61, but all cultures were negative for V. fetus. Lambs were not obtained from the other five ranches which had been on the experiment and there was no reason to believe that they had any disease problem.

A study was made in cooperation with Utah to determine the role of the ewe as a reservoir of infection for V. fetus. Cultures of 197 placentas from 2 bands of sheep which had 15% abortions the previous year, failed to reveal any Vibrio fetus. Also culture of aborted lambs was negative for Vibrio fetus. Twenty-two of 39 aborted lambs were cultured. Cultures of the bile of 18 ewes, whose placentas were infected with V. fetus the previous year, were found negative for V. fetus. In a field experiment involving 4,055 ewes, both yearlings and old ewes, there were about 2 to 2½ percent abortions in both the vaccinated and non-vaccinated ewes.

One of two herds vaccinated with Vib-Vac had a serious outbreak of vibriosis (rate of abortion 14.3%). Vibrio organisms were isolated from a ewe vaccinated the previous year. No vibrio organisms were isolated from the other herd which had an abortion rate of 1.7%.

About one third of the sheep tested from four different herds excreted an agent of the psittacosis-lymphogranuloma group but no vibrio organisms in the feces. This agent caused abortion in pregnant ewes identical to the picture of enzootic abortion.

Characteristic lesions in virus infected lambs and placentas were found which are distinctly different from lesions described in vibrio infected lambs and placentas.

Physiologic studies indicated that a given vibrio organism may have progeny with different physiologic properties when passed in ewes. Physiologically different strains were isolated from aborted lambs of the same herd.

D. Viral Ulcerative Dermatosis

In 1961, in cooperation with the Colorado Agricultural Experiment Station, success was achieved in isolating and culturing a viral agent from tissue exudates collected from a naturally occurring case of ovine ulcerative dermatosis (U.D.). After 10 serial passages on tissue culture, the culture fluids produced lesions typical of field cases of ulcerative dermatosis. The virus of contagious ecthyma (C.E.) was also cultivated on bovine kidney cell monolayers. The 2 viruses, when inoculated into scarified skin of lambs, produced lesions which could not be differentiated grossly or microscopically. The several physical properties of the two agents which were studied were found to be similar and did not produce a criteria for differentiation of the two diseases. The only distinguishing feature observed for the two diseases is that lambs which have become refractory following repeated inoculation with one agent were found to be susceptible to the other agent.

The experimental work completed in 1960-61 indicates that both ulcerative dermatosis and contagious ecthyma are caused by viral agents which can be propagated on bovine kidney cell cultures. No difference in the properties of the two viral agents were detected by the methods employed. Immunologic studies suggest that the etiological agents are poor antigens, or that the methods employed were not suitable for detecting antibodies. The studies further indicate the two viruses are closely related but not antigenically identical.

In 1962, the investigation of ulcerative dermatosis continued with 1) studies on the cultivation of UD virus on tissue cultures, immunology, and properties of the virus being repeated, using a new source of infectious material to see if the results could be duplicated; 2) attempts made to reproduce lesions on the external genitalia of young rams, using exudate from field cases of the disease.

Outbreaks of UD are being sought through the Wool Growers Association, county agents, and practicing veterinarians in Colorado and southern Wyoming, as a source of new material for further investigations.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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AREA NO. 4 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF HORSES

Problem. Currently there are about 3,250,000 horses in the United States, valued at approximately $660 million. About 1 million of these are draft animals. Considerable numbers of horses and mules are still required for work on cattle ranches and as pack animals. The annual overall value of the horse industry has been estimated at about $1.5 billion. No federal funds are presently available for research on equine diseases, although the horse may be an important link in epizootiology of animal diseases in general. Except at the Kentucky Agricultural Experiment Station, very little sustained research on diseases of horses has been accomplished in the United States during the last 20 years.

USDA PROGRAM

The Department is doing no research work on infectious and non-infectious diseases of horses. No line projects are in effect as of this date. African horsesickness, a highly fatal disease of equines, that was confined to Africa until recently, is presently causing serious losses in the Middle East and parts of Asia. In order to be prepared in the event of introduction of the disease into the United States, the Plum Island Animal Disease Laboratory has obtained African horsesickness viruses and antiserums from South Africa. These materials are thus directly available for diagnostic and vaccine studies, should the need arise.

The Federal scientific effort devoted to research in this area shows no professional man-years. P.L. 480 funds have been made available in Turkey for research on Gasterophilus pseudo-hemorrhoidalis (equine parasite) in Turkey; its distribution, life cycle, economic importance, treatment and control.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Agricultural Experiment Stations in 1961 reported a total of 3.2 p.m.y. divided among subheadings as follows: abortion in mares, pneumonia in young foals and new viruses 2.5 p.m.y., causes and pathology of lameness 0.1 p.m.y., and equine encephalomyelitis .6 p.m.y. Kentucky is working on new viruses, abortion-producing agents, and pneumonia. Maryland is studying the environmental factors conducive to production of equine encephalomyelitis, and more accurate tests for laboratory identification of the disease are being compared and improved. At Texas and Kentucky, detailed studies are being made on the pathology of bones, tendons, muscles, and joints involved in lameness in order to develop a scientific basis for the causes of lameness and to provide improved methods of treatment.

Industry and other organizations sponsor private research, research fellowships, and investigate and develop biological and pharmaceutical products of value to the horse industry.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

No research work is being done on infectious and non-infectious diseases of horses. There are no line projects in effect. The viruses and antisera for African horsesickness are being held available at the Plum Island Animal Disease Laboratory.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

None

AREA NO. 5 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF POULTRY

Problem. Annual losses from infectious and non-infectious diseases of poultry, exclusive of parasitisms, are estimated to be at least $200 million. Continued and expanded basic and applied research are essential to aid in reducing these losses, which inevitably affect cost to the consumer. Added to the initial losses from mortality, reduced weight gains, poor feed utilization, decreased egg production and lowered quality are the final losses occasioned by condemnations at dressing plants. Since institution of compulsory inspection for interstate movement of poultry and poultry products, overall condemnations because of disease have skyrocketed. The problem is to keep abreast of changing conditions in the field, which present increasingly complex problems requiring basic information.

USDA PROGRAM

The Department has a long-term program involving biochemists, microbiologists, pathologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of poultry. Research is being conducted on the diseases at the following locations.

The Federal scientific effort devoted to research in this area totals 31.4 professional man-years. This effort is applied as follows:

Ornithosis 5.1 at the National Animal Disease Laboratory, Ames, Iowa, and under cooperative agreements with the Universities of California and Minnesota, and the Agricultural Experiment Stations of Oregon and Texas.

Salmonellosis 3.0 at the National Animal Disease Laboratory, Ames, Iowa.

Pasteurellosis 2.0 at the National Animal Disease Laboratory, Ames, Iowa.

Chronic Respiratory Disease Complex 16.7 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the Agricultural Experiment Stations of Connecticut, Delaware, Georgia, Maryland, Massachusetts, New York, North Carolina, Texas, Virginia and Wisconsin, and with the University of Minnesota. A basic project on chronic respiratory disease is in progress at the Hebrew University, Jerusalem, Israel, under a P.L. 480 Grant with funds equivalent to $29,189 over a 3-year period.

Newcastle Disease 4.2 at the National Animal Disease Laboratory, Ames, Iowa, the Southeast Poultry Research Laboratory, Athens, Georgia, and under cooperative agreements with the University of Maine and the Wisconsin Agricultural Experiment Station.

Bluecomb 0.1 under contract with the University of Minnesota, St. Paul.

Leukosis 0.3 under cooperative agreement with the Regional Poultry Research Laboratory, USDA, East Lansing, Michigan.

Foot-and-Mouth and Other Exotic Diseases of Poultry 1.0 at the Plum Island Animal Disease Laboratory.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 58.6 professional man-years divided among subheadings as follows: Ornithosis 1.7; Salmonellosis 3.0; Chronic Respiratory Disease Complex 20.1; Newcastle disease 4.9; Infectious Bronchitis and Laryngotracheitis 2.4; Other diseases (hepatitis-synovitis syndrome, avian encephalomyelitis, aortic rupture, leucosis, staphylococcosis) 26.5.

Industry and Other Organizations are conducting studies to determine the efficacy of the pharmaceutical and biological products in the control of poultry diseases. Basic research on the various causative agents of poultry diseases has normally been completed essentially before these companies obtain the disease agents and undertake studies in relation to their respective products. Their objectives are two-fold - to aid in poultry disease control and to make a profit from their business. A number of these companies make grants to State Universities. A considerable number of National and State poultry organizations support research on poultry diseases through grants to State Universities and Experiment Stations.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Ornithosis

In 1961-62, the recently opened facility at the National Animal Disease Laboratory, Ames, Iowa, did not become suitable for conducting investigations on this highly dangerous disease of man, turkeys, and psittacine birds.

In 1961, in cooperative work with the University of California at Davis, a bacterium (Herrellae species) of low pathogenicity for humans was found to yield a soluble antigen which fixed complement in the presence of antibodies to ornithosis. The bacterium yield identical titers in human sera by the DCF test. The bacterial antigen had a high degree of correlation in detecting ornithosis antibodies in over 100 turkey sera. The soluble antigen was extracted from the Herrellae bacteria by boiling for 2 or more hours. It was extracted with acetone and ether from a boiled culture of the organism but could not be obtained from the raw bacterial suspension. Absorption experiments indicated that the antigenic components of the ornithosis agent is composed of substances common to ornithosis and the Herrellae bacteria.

In 1962 at the University of California laboratory, human serums of subjects suspected of having ornithosis or lymphogranuloma venereum were tested with the ornithosis and the Herellea-like bacterial (BA-16) antigens. Serums having high titers with the Texas ornithosis antigen also reacted with an antigen prepared from the bacterium.

A comparative study between the BA-16 strain of Herellea and three additional strains of Bacterium anitratum shows that there are antigenic differences to anti-ornithosis and anti-Herellea serums. One strain of B. anitratum which differed biochemically from the Herellea bacteria showed no cross-reaction with anti-ornithosis and anti-Herellea serums.

The Herellea-like strain of bacteria are highly pathogenic for guinea pigs when inoculated intraperitoneally and for chicken embryonating eggs, but was not pathogenic for turkeys. High titer complement fixing antibody serums could be prepared in turkeys following hyperimmunization.

A mouse-lung cell culture has been carried through 25 generations. The C-1 strain of ornithosis virus produced demonstrable elementary bodies and a cytopathogenic effect in the line cell in 24 to 48 hours that could not be demonstrated in the HeLa culture within 120 hours.

In 1961, in cooperation with the University of Minnesota, epidemiological studies revealed that the ornithosis agent was widespread in turkey flocks in some north central States. Examinations of sparrows in areas where the disease is enzootic failed to incriminate these birds as reservoirs of the agent. No virus isolations were made from turkey flocks showing serological evidence of ornithosis. The development of the DCF test for ornithosis has made available a practical diagnosis procedure in the study of this disease. Chlortetracycline proved effective in treatment of breeder flocks.

In 1962 at the University of Minnesota, serological studies continued to show a number of north central States have turkey breeder flocks showing ornithosis infection (6% to 23% of the flocks tested). The infection is of low virulence and no outbreaks have occurred, nor have virus isolations been made. Search for a reservoir of the agent has not been successful. Basic studies have been conducted to improve, simplify, and make the direct complement fixation diagnostic test more specific. Antibiotic treatment failed to show a direct effect on the diagnostic response of infected turkeys as compared to uninfected turkeys.

In 1961, cooperative work with the Oregon Experiment Station on natural reservoirs of the ornithosis agent failed to show that gulls and other free-flying birds were carriers of the agent in areas where the disease had broken out in turkey flocks sporadically for several years (1401 specimens were examined). Studies with earthworms failed to reveal them as probable virus reservoirs of the agent, even on infected premises. Susceptible turkeys did not develop infection when placed on contaminated litter. Infection was transmitted from clinically ill to susceptible turkeys through litter contact but not by aerosol.

In 1962, at the Oregon Station, six ornithosis isolates from turkeys, chickens, and sea gulls were studied in relation to their antigenic and pathogenic characteristics. The isolate from the sea gull was the most toxic for turkeys and produced mortality and severe lesions. One of the agents of chicken origin was the least toxic and apparently was incapable of infecting turkeys by the intramuscular route. This isolate would infect turkeys when inoculated into the air sacs, but it did not produce mortality.

The antigenicity of each strain in relation to turkeys was determined at 21 days post-inoculation based on the serological response to the indirect complement fixation test. The highest antigenic response was obtained with the gull strain of virus.

A transitory reaction to Mycoplasma gallisepticum-agglutinating antigen was produced in turkeys inoculated with ornithosis-infected mouse tissue. Experimental indirect complement-fixation (ICF) and agglutinating antigens were produced with the standard ornithosis viruses. The antigens were low antigenically and not satisfactory for the ICF test. The agglutinating antigens were too sensitive and gave a high percentage of false positive reactions.

In 1961, in cooperation with the Texas Agricultural Experiment Station, the histopathology of calves infected with the virulent and mild strains of ornithosis were studied. The lesions of the virulent strain were similar to those of sporadic bovine encephalomyelitis, whereas the mild strain produced no lesions.

A calf was used to produce antibodies for the complement-fixation (CF) diagnostic test to solve the problem of a source of positive serum. Turkey flocks were surveyed for infection and one flock detected. Virus was isolated from this flock.

Drug dosage was found not as important as time of therapy. Preliminary work shows that tylosin is an effective drug against ornithosis. Under experimental conditions 2 grams of tylosin per gallon of drinking water for 7 days was ineffective in ridding turkeys of the ornithosis agent, but 0.25 grams of tylosin per gallon of drinking water, administered for 3 weeks, was effective in removing the ornithosis agent from infected turkeys.

DNA derivatives, but not RNA derivatives, have the ability of overcoming the effect of aureomycin in tissue culture systems.

Adult sea gulls and sparrows were infected to determine their ability as potential reservoirs. Neither of these species developed the carrier state. Thirty-nine species of wild birds representing 278 blood samples were collected and tested for evidence of antibodies. One Foster tern had sufficient reaction to indicate previous infection.

In 1962, at the Texas Station, continued research revealed the agent of ornithosis caused abortion in 7 pregnant ewes and death in 2 non-pregnant ewes. Pigs were refractory to the agent of ornithosis when inoculated by various routes. The agent of ovine virus abortion caused some low serologic titer rises in turkeys but no lesions or signs, and virus was not isolated. Fractionization study of positive turkey sera shows the antibody to be of high molecular weight. There is an apparent difference between the ICF, Benedict DCF, and Broomfield DCF tests in the antibody measured.

DNA was effective in overcoming the effect of chlortetracycline but certain derivations or combinations of derivaties were only partially effective. Virus was not isolated from turkey eggs collected after inoculation of turkey hens with a mild strain (Minnesota) of ornithosis.

B. Salmonellosis

In 1961-62 at the National Animal Disease Laboratory, Ames, Iowa, insufficient staff and equipment precluded accomplishment of progress for a report.

C. Pasteurellosis

In 1961, at the National Animal Disease Laboratory, Ames, Iowa, research on this disease resulted in the following findings: A killed fowl cholera vaccine that had maintained a high degree of immunity for 1 year in chickens raised under experimental conditions, failed to prevent an outbreak of acute fowl cholera in turkeys raised under field conditions. Studies showed immunogenic differences between the vaccine strain of Pasteurella multocida and the strain isolated from the turkeys. A vaccine prepared with one type did not stimulate immunity against the other. A bivalent emulsified killed vaccine gave good protection in chickens for 37 weeks (duration of test) against both types. A bivalent, aluminum hydroxide adsorbed killed vaccine did not give satisfactory protection.

Serological tests showed that the two strains were different serotypes (Little and Lyons' classification). Serotype 1 fermented dulcitol but not xylose and was virulent for 16 and 45-week-old chickens. Serotype 3 fermented xylose but not dulcitol and was virulent for 16-week-old turkeys and 45-week-old chickens. However, 16-week-old chickens were more resistant to this type.

The biochemical and serological characteristics of 84 strains of P. multocida from 17 different States and the District of Columbia were similar to either strain X-73 or P-1059.

In 1962, at the National Animal Disease Laboratory, investigations of this widespread poultry disease gave the following results: 1) Passive immunity tests in mice, using hypered immune rabbit serums, did not differentiate between the two different immunogenic strains of P. multocida associated with fowl cholera. Therefore, the passive immunity test, which is often used to classify P. multocida, is of questionable value since it does not differentiate between these two immunogenic antigens. 2)Active immunity tests in mice, using an

emulsified vaccine, did not stimulate antibody production in mice to the degree that mice could be substituted for chickens when testing efficacy of emulsified fowl cholera vaccines. 3) Different strains of P. multocida of the same antigenic type can produce different pathology in chickens. One strain produced an acute septicemia, whereas another strain produced facial swelling and respiratory lesions. However, the septicemic strain when used in a vaccine prevented both types of disease. 4) In efforts to characterize antigenic components of related strains of P. multocida and to determine the chemical basis of virulence, host specificity and immunogenicity, hyperimmune rabbit serum and P. multocida antigen were produced from 3 different strains of P. multocida.

D. Chronic Respiratory Disease-Complex.

In 1961, at the National Animal Disease Laboratory, Ames, Iowa, research on this disease of poultry was begun late in the year - about July 1 - and the following report is for that period through June 30, 1962.

In 1962, at this Laboratory, results thus far show that all lyophilized cultures were viable after three years. Two strains were completely killed after freezing and thawing 5 and 7 times, whereas most strains survived the freezing and thawing 12 to 15 times. Only a few strains survived freezing and thawing 20 times. Cultures stored at -65°C showed a decrease of about 50 to 65 percent in viable count after one month. All cultures stored at -30°C survived 12 to 18 months. The cultures stored at 5°C survived from 20 to 60 days depending on the strain.

It has been observed that 0.85% sodium chloride solution is rapidly toxic to all strains of PPLO. The NaCl appeared to be equally toxic to 24-, 48-, and 72-hour cultures of the same strain. Buffering the saline solution with 0.02 N sodium phosphate reduced the toxicity. The addition of 1 ml. of growth medium to a liter of saline solution reduced the toxicity to three strains of non-pathogenic PPLO but enhanced the toxicity to two strains isolated from chickens affected with CRD.

The PPLO strains under study were purified to eliminate the possibility that bacterial cells may have been carried along in a dormant form in the PPLO cultures. Experiments to derive bacterial forms by culturing the PPLO in media at various pH levels, in media containing sub-optimal amounts of horse serum, and in medium containing no horse serum, were unsuccessful. In medium containing 5 percent yeast extract, however, two of seven PPLO strains showed bacterial cells in the 3rd and 5th transfers, respectively, and two others showed changes on solid medium in which the characteristics typical of PPLO were lost and the colonies appeared to be tiny bacterial colonies. Smears from these colonies, however, showed no bacterial cells.

Microbiological examination of turkeys showing signs of airsacculitis infections and sinusitis resulted in the isolation of Mycoplasma, Pasteurella multocida, and Escherichia coli. This re-emphasizes the importance of studying airsacculitis as a disease complex rather than as a specific disease entity.

Gross examinations of turkeys experimentally infected with Pasteurella multocida revealed lesions indistinguishable from those often observed in field cases of airsacculitis. This indicates the need for study of the lesions produced by certain strains of P. multocida and a comparison of these lesions with those produced by other agents and by combinations of agents associated with the air-sacculitis complex.

In 1961, personnel at the Animal Disease and Parasite Research Division's Southeast Poultry Research Laboratory, Athens, Georgia, began work in June in facilities of the Georgia Poultry Disease Research Laboratory at Athens. (Construction of the Division's laboratory began in April.) Accomplishments are combined with the 1962 report.

In 1962, research at this Laboratory include 1) Development of a method for preparation of M. gallisepticum (PPLO) diagnostic antigen; 2) Successful hyperimmunization of rabbits and chickens with M. gallisepticum for a source of specific antiserum; 3) Initiated field studies with several large commercial chicken growers to compare liveability, feed conversion, and other health factors in broilers from parents free of M. gallisepticum; 4) conducted basic studies on infectious bronchitis and Newcastle disease viruses, important complicating etiologic agents of the CRD syndrome.

In 1961, in cooperative research with the Agricultural Experiment Station of Connecticut, it was found that pathogenic cultures of the chronic respiratory disease agent, PPLO, placed on cardboard and eggshells failed to survive 48 hours at temperatures of 4°C, 25°C, and 37°C. Coliform organisms, closely associated with air sac infection in chickens, showed a wide variation in their virulence for chickens, and to belong to different serologic types within the pathogenic group.

Pleuropneumonia-like organism (PPLO) antigen for the diagnosis of the disease in chickens was not suitable for use in turkeys. Very high levels of antibiotics were necessary for control of CRD in chickens (1000 grams per ton of feed). Immunization of replacement stock at a young age (6 weeks) by intranasal exposure to pathogenic Mycoplasma gallisepticum (PPLO) seems promising. An antigen for PPLO diagnosis in chickens is now produced on a commercial basis.

In 1962, the Connecticut Station produced and distributed PPLO chicken antigen for testing chicken whole blood or serum for antibodies to Mycoplasma gallisepticum to 24 Stations and 11 countries. A survey of the incidence of flock infection to PPLO indicates a high rate of flock infection in Connecticut.

Production of a breeder flock of 14,000 chickens from PPLO-infected parents has been accomplished by testing with PPLO antigen, removal of infected hens, and practicing rigid security management. Antibiotic therapy of PPLO-infected birds stressed by other respiratory infections, seemed to favor use of combinations of antibiotics over one at a time. Active immunization studies with living PPLO are continuing.

In 1961, cooperative work with the Delaware Agricultural Experiment Station yielded these interesting results: 1) The amount of antibiotic entering a warm egg from a cool dip solution is dependent among other things upon concentration of antibiotic in the dip solution. Concentration of 600 and 800 ppm of erythromycin were superior to 400 ppm both in amounts and number of eggs showing antibiotic activity. 2) Of several new drugs tested, none proved to be superior to tetracycline antibotics fed as potentiated diets. The antibiotic, aureomycin, was not detected in eggs from chickens receiving 200 and 400 gram levels of antibiotic in the feed but was present in eggs when the level was raised to 1,000 or more grams per ton. 3) The USDA Killed Newcastle Disease vaccine gave 91% and 77% protection against challenge with the GB strain as compared to 20% and 19% protection afforded by the B-1 strain of vaccine. Compared to a tissue culture living vaccine, its use in broiler flocks resulted in less condemnations from air sac infection at slaughter.

In 1962, the Delaware Station studied the mechanics of treating hatching eggs with antibiotics for the control of ovarian transmission of PPLO. Various factors, including concentration and form of drug, temperature differential, time in solution, and influence of additives, have been studied to determine their influence on the egg dipping process. The results obtained provide fundamental information for the practical application of this procedure. Investigations of other methods of treating hatching eggs have been conducted and the results indicate that spraying of eggs is not satisfactory, while the application of vacuum or a combination of vacuum and spraying offers an additional means of treatment.

Contamination of eggs with organisms not susceptible to the drugs used in the treatment process is a constant problem and constitutes a major deterrent to practical use of these methods of control. Several new compounds, screened for their efficacy in treating experimental air-sac infection, failed to show superior results to those obtained with the tetracycline antibiotics potentiated by low calcium diets.

In 1961, cooperative work at the Georgia Agricultural Experiment Station on technics for diagnosis of PPLO and factors influencing the course of the disease, showed that the fluorescent antibody technic was not practical for diagnostic purposes. The hemagglutinating activity of pathogenic avian mycoplasma apparently is located in an acetone soluble fraction of the cell membrane. Experimental PPLO and E. coli infections do not directly influence vitamin A metabolism in the chick. Vitamin A levels in the ration do not affect the course of PPLO or E. coli infections in the chick except in severe avitaminosis.

Critical surveys in several broiler-producing areas have definitely established that variations in management and environmental factors, together with the use of live virus poultry vaccines, or concurrent natural outbreaks of other diseases, greatly influence the incidence and severity of air sac infection in chickens and the subsequent increase in condemnations when these birds are slaughtered for food.

In 1962 the Georgia Station conducted critical studies on the prophylactic and therapeutic value of antibiotics for prevention and treatment of CRD. Tylosin water medication of breeder flocks did not favorably influence the incidence of air sac lesions. Although injection of 10 mg. gallimycin or dihydrostreptomycin into day-old commercial broiler chicks as a preventative medication appeared to have some beneficial effects, in the main it did not justify labor and medication costs. Low levels of toxic fat ingredient (20%) fed to broilers for the first 6 weeks, as well as for the full production period 9 weeks) markedly influenced the condemnation rate due to severe air sac disease without invoking a clinically recognizable form of the disease. An extensive field study on the effect of preventive medication of broilers with furazolidone or chlortetracycline revealed a slight difference in favor of chlortetracycline in regards to mortality, feed conversion, condemnations, cost of medication and cost of production per pound.

In 1961, at the Maryland Agricultural Experiment Station, cooperative experimental research was conducted on growth requirements and serological classification of pathogenic M. gallisepticum (PPLO). This basic work afforded a sound approach to field problems.

In 1962 at the Maryland Station, work on experimental PPLO diagnostic antigens showed a prevalence of certain serologic types of PPLO in the State, differing somewhat from the types found in most States.

In 1961, at the Massachusetts Agricultural Experiment Station, cooperative research on CRD was conducted on several phases of the problem in chickens with the following results:

Cultural studies: Fermentation reactions can be used to separate the avian PPLO into two groups - fermenters and nonfermenters. Members within the fermenting group did not ferment the same carbohydrates. Further differentiation on the basis of carbohydrate fermentation was not regarded as reliable.

Transmission studies: Transmission of CRD to susceptible birds by contact exposure with premises previously occupied by CRD-infected birds was not successful in the single experiment that was conducted.

Serological studies: Studies of 19 avian PPLO were done in two phases. In the first phase, antiserums were obtained from rabbits after 6 to 10 inoculations and the tube agglutination test was used to classify the PPLO. The results showed that the PPLO fell into eight serotypes.

In the second phase of these studies, antiserums which would inhibit growth of the homologous PPLO (several strains required as many as 40 inoculations) were produced. The slide agglutination test was found to be of no value for classifying the PPLO. The tube agglutination and the growth inhibition tests were acceptable but neither alone was completely reliable. In these studies, seven serotypes were encountered and found to be related.

Response of CRD to medication. In four trials conducted in 6 to 7-week-old chickens, the value of chlortetracycline and oxytetracycline incorporated in low calcium diets was determined for the control of experimental CRD complicated with E. coli. Noninfected birds made the most favorable weight gains. The poorest weight gains were made by the infected-untreated groups. The infected-untreated groups also revealed more extensive and more severe involvement in the air sacs than did the treated groups. Medication did not appear to reduce the agglutinin response or the recovery rate of the agent from the trachea.

Control and eradication of CRD: a) Antibiotic egg dipping. Investigations to determine the effectiveness of destroying the CRD agent in the egg by dipping in erythromycin solution have indicated that bactericidal activity is observed within certain limits. b) Testing of flocks. The identification of CRD-free flocks with the serum-plate agglutination test and the establishment of additional flocks with progeny from negative flocks appear promising.

In 1962, the Massachusetts Station continued the investigations and reported these results:

Properties of the agent (viability studies): The viability of two strains of Mycoplasma gallisepticum (Hy and S6) is influenced markedly by temperature and the nature and quantity of materials in which the PPLO are suspended. In the various materials tested at temperatures above 0 C, the viability varied from 1 day to 120 days; at -20 C the PPLO were maintained for many months.

Transmission studies: Transmission of CRD to susceptible birds by contact exposure with premises previously occupied by CRD-infected birds was not successful. Two-year-old hens that had undergone a natural outbreak of the disease at an early age did not transmit the disease to susceptible birds by cohabitation or aerosol. Birds with active respiratory signs following experimental CRD-inoculation transmitted the disease to susceptible chickens by direct contact while transmission by aerosol was not successful.

Serology and immunity: Turkeys and chickens vaccinated with erysipelas bacterin produced a transient nonspecific serological response to PPLO serum-plate antigen. Marked resistance to challenge inoculation with a highly pathogenic CRD strain was demonstrated in chickens that have survived a natural outbreak of CRD. A satisfactory immune status was not produced in chickens by vaccination with an attenuated CRD strain.

Response of CRD to medication: The performance of chickens experimentally inoculated with CRD and E. coli followed by therapy with chlortetracycline and oxytetracycline, alone or in combination, potentiated by a low calcium diet, was superior to that of infected-nontreated chickens.

Control and eradication: CRD-free stock can be reproduced and maintained if adequate sanitation and management practices are observed.

In 1961 cooperative work at the University of Minnesota on Mycoplasma gallisepticum (PPLO) infection of poultry was directed to the disease in turkeys and resulted in these findings: 1) Field investigations strengthened the idea that airsacculitis is caused by a multiplicity of conditions, but infectious sinusitis does play a major role in its cause. 2) Studies tend to indicate that temperature and humidity may play a role in increasing the incidence of airsacculitis in a turkey flock. 3) A method for the production of an S_6 type of PPLO diagnostic antigen in large quantities was developed. 4) The Infectious Sinusitis Control Program was intensified by shifting to a 100% test and placing more stringent controls on the hatcheries and growers.

In 1962, at the University of Minnesota, studies were continued on the problem of PPLO in turkeys with the following results: 1) a satisfactory serum plate antigen has been produced in sufficient quantities to be available for an extensive pilot control program. 2) The serum plate, tube agglutination and HI tests gave comparable results as flock detection tests for infectious sinusitis. 3) The classification of PPLO isolates from turkeys on the basis of serological, pathological, and biological characteristics has been helpful in determining the role of various PPLO serotypes in airsacculitis. 4) Field investigations of farms experiencing excessive condemnations from airsacculitis indicated that infectious sinusitis was the major cause of high condemnations. 5) Air sac lesions in day-old poults appears to be a very common problem, and the role of "N" strain of mycoplasma in causing this problem is under investigation. 6) Investigations on a fryer-roaster turkey farm indicate the incidence of air sac lesions of both interclavicular and abdominal air sacs may be high, but condemnations may be low. The role of the "N" strain and other microorganisms in producing this residual exudate is under intestigation. 7) The intensified breeder flock inspection program and experimental infectious sinusitis control program has involved over 700 breeder flocks. The results indicate a very low incidence of the S_6 type in the breeder flocks. The progeny from these flocks are being critically followed during the growing period. Seven flocks have been examined from start to slaughter. The condemnation rate in these flocks has been extremely low, but the incidence of air sac lesions of both interclavicular and abdominal air sacs has been extremely high. A variety of organisms have been isolated, such as E. coli, aspergillus, Pasteurella, Salmonella.

In 1961, at the New York Agricultural Experiment Station, cooperative research was directed toward technics to prevent egg transmission of PPLO. Studies showed that dipping PPLO-infected eggs in erythromycin solutions - 400 to 1500 parts/million, reduced but did not eliminate the infection under experimental conditions. Under commercial conditions, 900 parts/million as a dip, without pre-warming the eggs, markedly reduced the condemnation rate and yielded significantly fewer serologically positive birds at 9½ weeks. Under experimental conditions, tylosin was superior to erythromycin as a dip solution for eggs infected with PPLO (S_6).

Subcutaneous injections of 25 and 5 mg of erythromycin and feeding of 350 gms aureomycin/ton with low calcium, lowered the egg transmission rate of PPLO infected hens. Complete elimination of the transmissions did not take place. Non-treated hens transmit PPLO through the egg to a decreasing extent as time passes.

Typical lesions of complicated CRD seen in field outbreaks were reproduced in the laboratory by the intratracheal inoculation of PPLO culture (S6), E. coli (pathogenic Virginia strains) and infectious bronchitis virus. For the most severe effect, the presence of PPLO was necessary with any combination of agents.

The prophylactic effect of serpasil, neomycin, furaltadone plus potentiated aureomycin, sulfalthoxypyrazine alone and with potentiated aureomycin was tested on birds inoculated with the combination of agents designed to produce complicated CRD. The last named drug combined with potentiated aureomycin gave excellent results.

Laboratory media adapted strains cannot be relied on to test efficiency of media for primary isolation of PPLO from tissues. Studies show, however, that fermentation reactions can be used to separate the avian PPLO into two groups, fermenters and nonfermenters. Members within the fermenting group did not ferment the same carbohydrates. Further differentiation on the basis of carbohydrate fermentation was not regarded as reliable.

In 1962, the New York Station continued the work on M. gallisepticum (PPLO) infection with these results:

A comparison of seven media for primary isolation of PPLO from chicken tracheas with a procedure involving preliminary enrichment in yolk sacs showed no marked superiority of one over the other.

Twelve distinct serotypes of avian PPLO have been determined by the colony inhibition technique of Edward.

Six sulfonamides combined with 500 grams per ton chlortetracycline and 0.5% terephthalic acid were screened for therapeutic effect against experimentally produced CRD. Two compounds gave best results - sulfaethoxypyridazine 0.0125% and sulfasoxazole 1.0% in the feed. Delaying medication of birds after infecting them reduced the efficiency of these compounds inversely in relation to the length of time of the delay. Benefit to contact-exposed birds was derived from medication but again delay of medication for longer than 2 days reduced or eliminated the therapeutic effect.

Dipping of chicken hatching eggs experimentally and naturally infected with Mycoplasma gallisepticum in concentrations of Tylosin over 800 parts per million for 5 to 10 minutes was highly effective in eliminating the infection.

Treatment of PPLO shedder hens with 15 mg. percorten had no effect on the transmission rate of PPLO in the egg.

Young chickens inoculated in the air sacs with relatively pathogenic strains of Mycoplasma gallisepticum did not shed PPLO into their eggs after a challenge inoculation when they came into production. Chickens inoculated with a mild pathogenic strain yielded 11% infected eggs. A control group produced 19% infected eggs.

In 1961 cooperative research with the North Carolina Agricultural Experiment Station was of a basic nature to determine antibacterial and antiviral activity of chicken serum. The susceptibility of fowl to E. coli inoculations were explored in an effort to more specifically determine the changes occurring following infectious bronchitis virus inoculation. Antibacterial activity of serum was studied relative to bronchitis inoculation to determine possible influence of bronchitis on susceptibility of E. coli inoculation using the colorometric method reported last year. This work is important in arriving at a better understanding of host defense mechanisms.

In 1962, cooperative research at the North Carolina Station on the defensive values of normal chicken serum for pathogenic microorganisms (E. coli) resulted in the development of a simplified procedure for electrometrically determining anti-E. coli activity of chicken serum. This test was employed in two experiments designed to determine the changes occurring in anti-E. coli activity of sera from young chickens inoculated with infectious bronchitis virus. A measurable reduction in anti-E. coli activity was observed in sera from birds inoculated with IBV, when compared with sera from non-inoculated, isolated controls. Observed changes closely coincide with results obtained in previous experiments employing in vivo challenges with E. coli.

Very critical histological studies indicate that the presence or absence of lymphofollicular lesions in the sinuses, tracheas, lungs and air sacs of turkeys which have been inoculated via the infraorbital sinus with a PPLO isolate is not a reliable criterion on which to base a diagnosis of pathogenicity of the isolate.

In 1961 the Texas Agricultural Experiment Station conducted cooperative research on PPLO in poultry and results indicated the hemagglutination (HI) test to be a more accurate diagnostic tool than the serum plate (SP) test, in experimental eradication of infectious sinusitis of turkeys of 36 breeder flocks (38,324 birds). The antibiotic spiramycin-adipate was extremely effective in treating IS, but was of little value in treating airsacculitis of turkeys.

Variant types of M. gallisepticum (PPLO) were discovered during the year's work. The control of PPLO egg transmission by injection of hatching eggs with a combination of tylosin tartrate, 50 micrograms, and dihydrostreptomycin, 5 milligrams, proved not feasible because of greatly reduced hatchability.

In 1962 the Texas Station continued its cooperative investigations with the following results:

When M. gallisepticum infected eggs were dipped in chilled antibiotic solutions, egg transmitted infection was markedly reduced and broilers so produced remained relatively free of infection during the growing period. Condemnation losses were significantly reduced in birds produced from dipped eggs, and birds so produced outweighed controls by 0.23 pounds. Techniques were developed by which uniform M. gallisepticum plate and tube test antigen could be produced in a simplified medium. The serum plate diagnostic test for infectious sinusitis has been successfully used in eradication of the disease from many hatchery supply flocks.

In 1961, at the Virginia Agricultural Experiment Station, cooperative research on CRD was conducted on basic and applied facets of the problem with these results: 1) PPLO do not readily mutate to increased resistance to furazolidone, tetracycline or chloromycetin. 2) S-6 type PPLO are not physiologically identical, i.e., they are inhibited by different concentrations of glucose and produce different degrees of acidity from identical amounts of glucose. It is possible that one medium will not be suitable for the isolation of all S-6 type PPLO. 3) S-6 type PPLO produce distinctive breast blisters. 4) A "PPLO-free" flock has been maintained for 5 consecutive years through the use of good management practices. 5) Vaccine viruses will greatly increase the contact spread of PPLO and the severity of the lesions. 6) Pathogenic strains of E. coli were isolated from feed, but all the coliforms were destroyed by pelleting. 7) The duration of susceptibility of respiratory disease infected chickens to E. coli lasts about 50 days.

In 1962, the Virginia Station directed the cooperative research toward certain stress factors which affect CRD, and other related problems, with the following results: 1) M. gallisepticum produced an experimental salpingitis in 64% of individuals observed following yolk sac or air sac inoculation of day-old chicks. M. gallisepticum could be recovered from affected oviducts until after the flock was in egg production (25 weeks). 2) An improved formula for the medium to propagate M. gallisepticum has been developed. 3) A "PPLO-free" flock has been maintained for 6 consecutive years through good management practices. 4) Thirty-six percent of 518 immature chickens, vaccinated with live pathogenic M. gallisepticum, were protected against small challenge doses of the organism. Similar vaccination of birds kept for laying had no significant effect on egg production, hatchability, or progeny. Egg transmission of the agent was not detected. 5) Lesions suggestive of those produced by Mycoplasma gallisepticum were produced following the inoculation of the B_1 strain of Newcastle disease vaccine by the air sac or aerosol routes. 6) Increasing the concentration of toxic fat, but not salt, in the feed increased the incidence of coliform infection following intravenous inoculation. Toxic fat-fed chickens made a poor defensive reaction against air sac inoculated E. coli. Furaltadone, but not specific antiserum, protected toxic fat-fed birds from air sac or IV inoculated E. coli.

In 1961 at the Wisconsin Agricultural Experiment Station, starting in May of that year, cooperative research was begun on the problem of airsacculitis in turkeys. The multi-discipline research approach included studies of etiologic agents and the impact of agrometeorological factors. This research resulted in the following statements concerning 4 flocks of turkeys from hatching to slaughter. 1) Airsacculitis is a major problem in the turkey-production area of Wisconsin. 2) There apparently is no single etiological agent which causes the majority of airsacculitis in the study area. A number of infectious agents, some in the presence of certain stressors, have in the past been shown to be a possible contributing factor to avian air sac lesions. None of these can be ruled out, at present, as a contributing factor to airsacculitis in this area. 3) It can be said that, if the present means of isolation are sufficiently sensitive, airsacculitis can occur in the absence of heavy

persistent infections with mycoplasma, ornithosis virus, or bacteria. 4) The continuous high-level feeding of antibiotics may be encouraging persistence of certain atypical forms of bacteria in the tissues of the poults and actually put the birds at a disadvantage in the host-parasite relationship. An agro-meteorological station was designed and built to continually record on tape environmental conditions inside and outside the building. The building is a typical "pole" type of the area and will house 400 turkeys. Preliminary determinations of carbon monoxide, carbon dioxide, and ammonia concentrations have been made.

In 1961-62, the work on CRD under PL 480 grant to the Hebrew University, Israel, was of a preliminary educational nature to the investigators.

E. Newcastle Disease.

In 1961, at the National Animal Disease Laboratory, Ames, Iowa, research work was done on different killed Newcastle disease (ND) vaccines with respect to different ratios of Amphojel to the macerated virus material prepared from embryonated chicken eggs (ECE), replacement of the ECE virus material with normal embryonated chicken eggs or amnio-allantoic fluid, and comparison of inactivation of virus material before and after the addition of Amphojel. Under the conditions of the experiment, vaccines of low virus material content, except or less than .5%, with the proper amount of Amphojel, were equally effective as higher virus material content vaccines. Normal tissue vaccines had little if any potency. Amnio-allantoic fluid vaccine compared favorably with ECE virus tissue vaccines. Inactivation of virus material after the addition of Amphojel indicates that the immunogenicity of the vaccine was reduced significantly as compared with similar virus inactivated before the addition to Amphojel.

Direct complement fixation tests on chicken serums within 1 hour of collection tended to be more reliable than on those that had been collected for longer periods or on those stored at 4°C for several days.

In 1962, at the National Animal Disease Laboratory, in studies on Newcastle disease a chicken anti-sheep hemolysin was developed for the complement-fixation system. This made possible the conglutinating complement adsorption study of avian serums because chicken complement cannot be tested with rabbit anti-sheep hemolysin nor can guinea pig complement be titrated with chicken anti-sheep hemolysins.

The conglutinating complement adsorption test (CCAT) for avian (chicken and turkey) sera was successfully developed using Brucella and ornithosis antigens. Initial trials with Newcastle disease (ND) antigen were negative.

Experiments designed to measure the relationship of embryo killing time for ND virus and ND vaccine antigenicity are in progress. Ten vaccines were each prepared with virus from embryos inoculated with different serial ten-fold dilutions (10^{-1} to 10^{-10}). The embryo death time increased after the 10^{-5} dilution and was negative in the 10^{-10} dilution, but the virus from the dead

embryo fluids in each dilution had similar end point infectivity titers ($10^{-8.5}$) and hemagglutination (HA) titers (1280). The vaccines have been injected into birds for serological and virus challenge immunity tests.

Newcastle virus was successfully grown in tissue culture through 10 passages in primary chicken embryo fibroblasts, resulted in maximal egg infectivity titers of 10^7 and HA titers of 320.

ADP recommended, killed Newcastle disease virus (NDV) vaccines presently being used in the field contain 45% macerated embryonated chicken egg virus (ECEV) and 50% amphojel. Studies of NDV adsorption on amphojel ($AlOH_3$) has shown that this ratio of amphojel to ECEV will adsorb less than 0.1 percent of the virus in the vaccine as measured by egg embryo infectivity tests. All live virus infectivity was not adsorbed at a ratio of 97.5% amphojel and 2.5% ECEV. A ratio of 80% amphojel and 20% ECE was required to remove all the hemagglutination units from the centrifuged vaccine supernatant.

Elution studies of virus from amphojel vaccine by serial washings with equal parts phosphate buffered saline and ECEV saturated amphojel were made to simulate the release of virus from amphojel in vaccinated birds. Negative HA tests were obtained from serial washings of ECE alone on the third wash, from amphojel saturated with ECE virus on the 24th wash, and from amphojel saturated with allantoic fluid virus on the first 7 washings and again on the 90th wash. These results indicate a correlation between virus concentration and/or purity and duration of virus adsorption on amphojel. Quantitatively a very low percent (1-2%) of virus could be washed off of amphojel and detected by the HA testing system. Consequently, a more accurate method of testing washings for virus must be developed.

In 1961, in cooperative studies with the Wisconsin Agricultural Experiment Station, Madison, a Newcastle disease virus (NDV) repository was maintained, and characterization studies of a number of virus strains were conducted. Strains of NDV were distributed under federal permit to biological houses and research institutions. The development of technics for diagnosis and characterization of NDV by various methods under varying circumstances is continuing.

In 1962, at the Wisconsin Station, an assay method for characterization of NDV was developed. It is sensitive and dependable, and is based on the ability of NDV to form plaques in a primary cell tissue culture growth medium. Factors affecting kinetics of growth and genetic markers by the use of inhibitors are being studied for their value in differentiation of NDV strains. It was found that NDV strains from Mexico, Mexico-MU, and Mexico-Sonora, are not as readily neutralized by antisera to B1, one of the standard vaccine strains. It takes a thousand times as much B. antiserum to neutralize the heterologous Mexican strains as it does to neutralize the homologous strain. The staff of the repository have assumed responsibility for planning a symposium on "Newcastle Disease Virus as an Evolving Pathogen." The purpose of the symposium is to achieve a better perspective of the research on the virus of Newcastle disease as a strain-complex.

In 1961-1962, at the University of Maine, Orono, a cooperative experimental field study on Newcastle disease was begun. The purpose was to determine the efficacy of a killed ND vaccine in controlling and eradicating the disease in a large breeder and broiler growing area, and to determine the effect of such a ND vaccination program on the incidence and severity of air sac infection. To date approximately 6.2 million doses of dead ADP type Newcastle disease vaccine has been used in the State of Maine with no known outbreaks of ND in vaccinated flocks. A natural outbreak in a broiler flock of ND was counteracted in the succeeding two flocks by vaccination with dead ND vaccine with no further trouble. The incidence and severity of air sac infection together with condemnations, were greatly reduced in these birds.

In 1961-1962 work on Newcastle disease at Pulawy, Poland, under a PL 480 Grant, successfully demonstrated that yolk contained antibodies of immune dams decreased the virulence of Newcastle virus when it was passaged through them at least thirty times. The same results were obtained when the virulent virus was passed thirty times through eggs from susceptible dams if specific antiserum had been introduced into the yolk sac prior to inoculation of the Newcastle virus. Parallel results were obtained by passing virulent Newcastle virus 20 to 30 times through immune 4 to 6-day old chickens, but not through immune 13 to 14-day old chickens.

F. Bluecomb in Turkeys.

In 1961-1962 investigations on this disease were begun under contract with the University of Minnesota. Suspected bluecomb infected turkeys from 28 commercial flocks have been subjected to study. Isolation of the causative agent by bacteriological, chicken embryo inoculation, and tissue culture technics has been successful in embryo inoculation in 12 instances. The agent has been passed by feeding and intraperitoneal injection of susceptible poults, and recovered from the intestinal tract 5 days after infection. The agent has been shown to be filterable, fairly resistant to antibiotics, and does not grow on non-living media.

G. Foot-and-Mouth and Other Exotic Diseases of Poultry.

In 1961, at the Plum Island Animal Disease Laboratory, the serological response of adult chickens to infective and non-infective preparations of two types of foot-and-mouth disease virus was studied. The peak of antibody following inoculation with infectious virus appeared approximately 28 days after inoculation. Low levels of complement-fixing antibody developed at 7 days after inoculation of non-infective virus. Chickens inoculated with infective tissue-culture virus and those inoculated with non-infective virus of the same type inactivated with acetylethyleneimine and beta-propiolactone, developed high levels of neutralizing antibody. Similarly chickens inoculated with formalin-inactivated A-119 virus produced in tissue cultures also developed high levels of antibody.

In 1962 no studies in this area were conducted at the Plum Island Animal Disease Laboratory

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AREA 6 - INFECTIOUS AND NON-INFECTIOUS DISEASES OF FUR ANIMALS, INCLUDING RABBITS

Problem. The business of raising fur animals, such as rabbits, chinchillas, mink, and foxes, in captivity encounters disease problems incidental to the confinement of such animals. These include viral, bacterial, parasitic, mycotic, nutritional, and hereditary diseases. The enteric disease-complex causes great mortality in commercial rabbit production. It destroys whole litters and commonly attacks all susceptible rabbits on a farm. The respiratory disease-complex, perhaps, is second as a cause of mortality. In severe outbreaks over 50 percent of adult animals may die. These two diseases cause great economic loss to the rabbit industry which produces an estimated 50 million pounds of meat annually and millions of dollars worth of rabbits for experimental purposes.

USDA PROGRAM

The Department has a continuing long-term program involving microbiologists and veterinarians engaged in both basic studies and the application of known principles to the solution of infectious and non-infectious diseases of fur animals, including rabbits. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 3 professional man-years. This effort is applied as follows:

Coordinated Field and Laboratory Studies, 1.0 at the U. S. Fur Animal Disease Research Laboratory, Pullman, Washington.

Enteric Disease-Complex of Rabbits, 0.5 at the U. S. Rabbit Experiment Station, Fontana, California.

Respiratory Disease-Complex in Rabbits, 0.5 at the U. S. Rabbit Experiment Station, Fontana, California.

Transmission of Infectious Diseases by Helminths, 1.0 at the U. S. Fur Animal Disease Research Station, Pullman, Washington.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 2.4 professional man-years devoted to diseases of fur animals. California studies are evaluating immunity in myxomatosis of rabbits. Connecticut and Washington are searching for factors causing Aleutian disease of mink. Studies at Washington are on virus enteritis in mink.

Industry and other organizations have been estimated to devote equivalent to 5 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Diseases of Fur Animals, including Rabbits.

Distemper. In 1961, at the Fur Animal Disease Research Laboratory, Pullman, Washington, an estimated number (probit analysis) of 2 and 32 Biological Units (BU) of embryo-propagated distemper virus (DV) immunized ferrets and minks respectively. The minimal number of BU needed to initiate a detectable immune response was not established. No quantitative relationship could be demonstrated between immunizing doses of attenuated virus and challenge doses of virulent distemper virus.

When ferrets were vaccinated with tissue culture origin modified live virus distemper virus vaccine, the onset of immunity was partially controlled by the strain of challenge virus utilized. When a "short incubation period" challenge virus was used, the animals were immunized three or more days after vaccination, whereas, when a "long incubation period" challenge virus was administered, all ferrets vaccinated one day or more prior to challenge were refractory to challenge.

In 1962 the interference phenomenon was observed between two living distemper virus (DV) variants in ferrets. The interfering variant was varying quantities (-3 to 30,000 EID_{50}) of chicken embryo-adapted DV and two concentrations (5 ferret LD_{50} and -1000 ferret LD_{50}) of virulent virus. The source of virulent virus was a ferret-adapted variant, which was characterized by a short incubation period. The logarithmic transformation of the incubation periods was normally distributed.

When the embryo-propagated variant was given at levels of 300 EID_{50} or more at intervals prior to, simultaneously, or shortly after the injection of 5 ferret LD_{50} of virulent virus, interference was observed. If the level of virulent virus was increased to -1000 ferret LD_{50}, the course of the natural disease was not altered. The larger the dose of attenuated virus, the shorter the interval required to develop resistance. At the ratio of -30,000 EID_{50} and 5 ferret LD_{50}, resistance occurred even if the interfering attenuated virus was given after challenge. This post challenge interval did not exceed 1/3 (3 days) of the incubation period of the virulent challenge virus.

Botulism. In 1961 it was determined that young mink from botulism-immunized females were successfully immunized with botulism toxoid as early as 4 to 5 weeks of age. Ferrets vaccinated with botulism toxoid were still susceptible after a 28-day interval when the toxin challenge was given by the intraperitoneal route.

Mink Virus Enteritis. In 1961 it was determined that mink can be immunized by low, but not high dilutions of feline panleukopenia virus administered by the oral route. Extremely high concentrations of FLV given to mink by the oral route may occasionally result in mild signs resembling mink virus enteritis (MVE) infection. Feline panleukopenia virus is transmitted with difficulty

within a closed colony of mink. Mink vaccinated with inactivated mink virus enteritis vaccines and living feline panleukopenia virus vaccines may shed the virus in their feces for a considerable length of time (at least 30 days). Raccoons may be carriers of mink virus enteritis and should be considered in control programs. Bolin's virus is not identical with feline panleukopenia virus.

In 1962 mink virus enteritis and feline panleukopenia were compared on a clinical, pathologic and immunologic basis. Cross immunization between feline panleukopenia and mink virus enteritis was accomplished in cats, mink, and raccoons. Clinical and pathological observations confirmed the similarity of both diseases in their natural hosts species. Both virus diseases seemed to produce swelling of both the nucleoli of epithelial cells of the intestinal mucosa when studied in their natural hosts; these changes may have diagnostic significance. Feline panleukopenia was passed through three serial mink and three alternate mink-cat passages without evoking mink pathogenicity.

Mink can be immunized against MVE by subcutaneous or oral inoculation with FLV. Mink inoculated with live FLV transmitted virus occasionally to uninoculated contact mink; this transfer was more readily accomplished by mink inoculated via the oral route than the subcutaneous route. Mink virus enteritis did not produce clinical disease in cats but persisted for nine, but not twelve days in the viscera when given parenterally. Mink virus enteritis transfer from cat to cat was accomplished by intraperitoneal injection of feline tissue suspensions.

Cats can be successfully immunized against feline panleukopenia using either live MVE or commercial inactivated mink virus enteritis vaccine. Raccoons were not readily infected with mink virus enteritis but the virus persisted for more than 12 days in raccoon tissue and was excreted with the feces. Mink recovered from experimental mink virus enteritis may excrete virulent MVE with their feces for at least 12 months, as demonstrated by inoculation of indicator mink.

Adaptation of FLV to laboratory animals as rabbits, guinea pigs, ferrets, chinchilla, mice and hamsters was unsuccessful. Attempts to propagate FLV in primary cell cultures of feline kidney, feline bone marrow, feline spleen, canine kidney, raccoon kidney, ferret kidney, and mink kidney were unsuccessful. Cultivation of FLV in serially propagated lines of canine kidney, human synovial cells (McCoy), and human carcinoma (HeLa) failed to produce evidence of virus multiplication.

Salmon Poisoning. In 1961 it was conclusively proven that the endoparasite Nanophyetus (Troglotrema) salmincola, contrary to the literature, is not "cleansed" from the migrating salmon when the salmon enter salt water. These parasites have been shown to remain alive for at least 3 years. Of greater importance Neorickettsia helminthoeca, the etiological agent of salmon poisoning, also remains alive for at least this length of time. An endpoint has not as yet been reached.

Hypergammaglobulinemia in Mink. In 1961 the electrophoretic patterns of normal and Aleutian-disease affected mink have been determined. The serum from diseased animals had increased total serum proteins, increased gamma globulin, and decreased albumin.

In 1962 more than 700 normal and Aleutian-disease affected mink serums have been examined. A constant finding was increased total serum protein, decreased albumin, and increased gamma globulin as determined by zone electrophoresis. A simple field test for Aleutian disease to detect changes in the serum proteins has been developed. A simple iodine test solution is added to the mink serum. In case of an animal affected with Aleutian disease, fine agglutinated particles appear after a short period of time. The results of the test appear to be very encouraging. On one ranch the losses were reduced by 75 percent. About 35,000 mink had been tested since the test became available early in 1962. This will become a standard ranch procedure; however, it will also be a valuable research tool.

Aleutian disease was experimentally produced in susceptible mink using formalinized tissue suspensions which were held for two weeks at 5°C. When this same tissue suspension was held for 40 weeks at the same temperature, the disease was not produced. These findings suggest that Aleutian disease may be caused by an infectious agent which is inactivated by formalin. Lesions in mink affected with Aleutian disease resemble many of those described for such diseases in man as periarteritis nodosa, lupus erythematosus and coupled with increased gamma globulin suggests that the mink may serve as a model for these important poorly understood diseases of man.

B. Enteric Disease-Complex of Rabbits.

In 1961, at the U. S. Rabbit Experiment Station, Fontana, California, the addition of a digestive enzyme based on proteolytic activity to the feed failed to lower feed conversion or to alter the enteritis complex. Two nitrofurans, nitrofurazone and furazolidone, used separately and in combination did not prevent the development of liver lesions in animals experimentally infected with Eimeria stiedae. When used in combination, the two nitrofurans had a detrimental effect on the life cycle by causing the parasites to produce many infertile forms in the bile tracts. The effectiveness of 50 mg/ton of zinc bacitracin on the enteritis complex, and isolation of members of the clostridia group, is being studied. A field outbreak of nest-box scours was caused by a Paracolon organism. A good response was noted to the oral feeding of a nitrofuran compound.

In 1962 the addition of 25 and 50/ton NF-180 reduced mortality from enteritis, lowered feed conversion, and increased average weaned weights. Continuous feeding, 8 - 12 months may have a detrimental effect. Data from successive mixes show loss of advantages over control lot, especially on the 25 grams level. A 4 - 6 months continuous feeding appears to be optimum. Zinc bacitracin incorporated at a 50 gm/ton level in the feed reduced mortality from enteritis and increased average weaned weights. Further work on both 25 and 50 gm levels are indicated. Continuous feeding of a ration containing

100 gm chlorotetracycline plus 50 gm oxytetracycline was more effective in reducing enteritis than when fed during age 35 to 45 days, the period when enteritis is at its peak. Stress of re-breeding does when the young are 10 days old, and weaning the fryers at 35 days of age, had no influence on enteritis mortality. Additional time is required to make usual weight gains in the meat pen.

Male animals developed on high levels of NF-180 showed delayed maturation of spermatozoa and atrophy of the seminiferous tubules. Basal cells of the testicle are not destroyed. Amprolium, a widely used water soluble coccidiostat, was not effective in controlling liver type coccidiosis. Shope's papilloma, a virus infection of the wild rabbit, has been found in domestic rabbits in San Diego and San Bernardino counties. Previously unreported, an intestinal species of coccidiosis has been recovered from the chinchilla. Sporulation of the oocysts have been completed.

C. Respiratory Disease-Complex of Rabbits.

In 1961, at the U. S. Rabbit Experiment Station, Fontana, California, myxomatosis was found in a species of wild California brush rabbit. Active and virus-producing lesions were found to last as long as 3 months and it was concluded that this was the reservoir of infection in California. White, subcutaneous absesses found on nest-box young were caused by staphylococcus infections. The organism was penicillin-resistant but sensitive to other antibiotics. Grieseofulvin, fed at a 10 mg/lb. body weight level, was effective in controlling laboratory infected and field cases of favus. Laboratory infections involved Trichophyton mentagrophytes, T. verrucosum, Microsporum canis, and M. gypseum. Respiratory infections, caused by Pasteurella multocida, were effectively treated with penicillin combined with streptomycin.

In 1962 a new, unreported manifestation of pasteurellosis is described. Initial infection is believed the result of a puncture injury or insect bite on the ear. Infections by Pasteurella pseudotuberculosis do not respond to medication by feed-grade or water-soluble furazolidone.

D. Transmission of Infectious Diseases by Helminths.

In 1962, at the Fur Animal Disease Research Laboratory, Pullman, Washington, naturally occurring definitive hosts of adult Troglotrema salmincola, include the bobcat and coyote. The release of cercariae in infected waters was shown to occur first in April with a peak release in June and July, and another peak in October and November, with cessation of release in December. Cercariae were not released in January, February, and March. We further demonstrated that metacercariae containing Neorickettsiae helminthoeca can remain viable in nature for 3 years. Preliminary evidence suggests that at least a 4-year longevity of metacercariae and rickettsae is possible. It has been shown that barriers to migration of snails infected with this fluke are possible and that the proper use of molluscacides can reduce the incidence of the vector snail. A naturally occurring disease immunologically distinct from salmon poisoning transmitted by Troglotrema salmincola, was observed again during this report period.

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AREA NO. 7 - MISCELLANEOUS INFECTIOUS AND NON-INFECTIOUS DISEASES OF ANIMALS

Problem. Included in this area of research are diseases such as vesicular stomatitis, which affects cattle, horses, swine, and man; poisoning by various plants, which differ in toxicity according to local conditions, and affect different species of animals in various ways; agricultural chemicals, such as herbicides and pesticides, which may produce poisoning in animals, especially if not properly used, and may also leave dangerous residues in the soil, feed, or animal body; tumors, including cancer, which affect all species of animals; bloat, a common, serious condition in cattle and sheep; and potential dangers of "fall-out" from nuclear testing or attack. Investigations of these diverse hazards to livestock and poultry require modern techniques as well as fundamental approaches through chemistry, pathology, physics, physiology, and other scientific disciplines. The problems are so complex, diverse, and numerous that it has been impossible to more than scratch the surface in probing for basic knowledge required for protection of the nation's livestock and poultry populations.

USDA PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, pathologists, physicists, and veterinarians engaged in both basic studies and the application of known principles to the solution of miscellaneous infectious and non-infectious diseases of animals. Research is being conducted at the designated locations.

The Federal scientific effort devoted to research in this area totals 18.0 professional man-years. This effort is divided among sub-headings as follows:

Incidence and Pathology of Tumors 1.0 at the National Animal Disease Laboratory, Ames, Iowa. A grant of P.L. 480 funds equivalent to $51,383 has been placed with the Veterinary Faculty, Ankara University, Ankara, Turkey, on etiologic investigation of bovine urinary bladder tumors due to enzootic bovine hematuria in Turkey and its relation to bovine papilloma agent.

Vesicular Stomatitis 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Components of Normal and Immune Serum 1.0 at the National Animal Disease Laboratory, Ames, Iowa.

Bloat in Ruminants 4.5 at the National Animal Disease Laboratory, Ames, Iowa, and through cooperative agreements with the California, Maryland, and Mississippi Agricultural Experiment Stations and with the New York State Veterinary College.

Preparedness for Diagnosis of Foreign Animal Diseases 2.5 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

Toxicology and Pathology Related to Insecticides 2.5 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, in cooperation with the Entomology Research Division.

Biochemical Effects of Agricultural Chemicals 0.9 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas, and through Cooperative Agreement with the Stephen F. Austin College at Nacogdoches, Texas.

Detoxication Mechanisms in Cattle and Sheep 0.5 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Cytological Responses to Antiparasitic and Other Agricultural Chemicals 0.5 at the ADP Field Station, Toxicology Laboratory, Kerrville, Texas.

Poisoning by Plants 1.1 at the Logan, Utah, Field Station and through cooperative agreement with the Utah Agricultural Experiment Station and through informal cooperation with the U. S. Plant Soil and Nutrition Laboratory of Soil and Water Conservation Service, Ithaca, New York. A grant of P.L. 480 funds equivalent to $56,746 has been placed with the Instituto Biologico, Sao Paulo, Brazil, on the study of plants of the State of Sao Paulo poisonous to domestic animals.

Toxicity of Herbicides and Herbicide-Treated Plants for Domestic Animals 1.0 at the Logan, Utah, field station with informal cooperation with the Utah Agricultural Experiment Station and the Crops Protection Branch of the Crops Research Division at Logan, Utah.

Alleviators and Diagnostic Tests for Plant Poisoning 1.0 at the Logan, Utah, field station through informal cooperation with the Utah State University, the Crops Research Division and the Forest Service.

The Susceptibility of Wild Animals to Foot-and-Mouth Disease 0.5 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 16.4 divided among subheadings as follows: Poisoning by Plants 3.1 at Arizona, South Dakota, Texas, Utah, Wyoming. Biochemical Effects of Agricultural Chemicals 3.0 in the North Central, Southern, and Western Regions. Bloat in Ruminants 6.3 at Iowa, Minnesota, and Wisconsin Agricultural Experiment Stations. Other Conditions-Pathology of Tumors, etc. 4.1 in all four regions.

Industry and other organizations are engaged substantially in research and development of agricultural parasiticides, herbicides, feed additives, insecticides, and pesticides that may be used in production of both feed and livestock, or may endanger livestock exposed to them through misuse, miscalculation, or accident. Aggregate expenditures by chemical and other companies in relation to these products probably are as great or greater than those in any other single agricultural market area. USDA, State stations, veterinary schools and colleges, and others are called upon from time to time for assistance or collaboration in evaluation or safety-testing of compounds that are potentially useful to the livestock industry. Samples of new, ostensibly useful and safe compounds are frequently proffered free of charge for comparative laboratory or field testing with other previously used materials. Such offers to USDA are accepted when testing is deemed both practicable and directly useful in the over-all program of research aimed at development of sound programs of prevention and control of disease of livestock and poultry. In its search for more effective compounds in the area of miscellaneous infectious and non-infectious diseases of animals, and in toxicological studies, it is estimated that industry devotes no less than 20 professional man-years per annum.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Incidence and Pathology of Tumors.

Work of ten years was closed on tumors at the Denver Animal Disease Laboratory during 1961. In 1962 the equipment, specimens, and that finished portion of the colored atlas of animal pathology with planned concise descriptions of 75 selected animal diseases was moved to the National Animal Disease Laboratory, Ames, Iowa, for the current work.

The results obtained so far during 1961 and 1962 under Public Law 480 with Turkey on investigations as to the cause of bovine urinary bladder tumors, associated with or due to enzootic hematuria, have indicated that spontaneous tumors of bovine urinary bladders produced papilloma in the skin of calves.

B. Components of Normal and Immune Serum.

In 1961 the following is a summary of the work done at the National Animal Disease Laboratory at Ames, Iowa.

The technique of separating high and low molecular weight agglutinins for Brucella by ultracentrifugation of serum layered over a density gradient sucrose solution (10 to 40 percent) was applied to 19 serum samples collected from 4 pregnant heifers in the interval of 15 to 63 days after exposure to virulent Br. abortus strain 2308. Within the period of 15 to 22 days after exposure, the serum agglutinins were predominately of high molecular weight (80 to 100 percent). The amounts of high molecular weight agglutins remained fairly constant in the period of 22 to 63 days after exposure. At about 30 days following exposure, significant levels of low molecular weight were evident, and by 63 days following exposure increased to the extent of constituting 70 to 94 percent of the total agglutinins.

Density gradient centrifugation studies were made on 80 serum samples collected from 20 heifers, 5 to 91 days after vaccination with Br.abortus strain 19. High molecular weight agglutinins constituted 86 to 100 percent of the agglutinins present in serums collected in the interval of 5 to 15 days post-vaccination. Relatively small amounts of low molecular weight agglutinins were found in serums collected from 8 animals in the interval of 17 to 91 days post-vaccination.

The heat inactivation test (65°C., 15 minutes) indicated a fairly close correlation between the percent heat labile agglutinins and percent high molecular weight agglutinins in the serums from the vaccinated animals. The titers obtained with the 56°C., 18-hour heat inactivation test of Hoerlein on serums from 16 heifers 5 to 10 days post-vaccination, were very similar to the titers obtained with the standard tube test.

In 1962 the M-2 glycoprotein of bovine plasma was prepared by the ammonium sulfate-carboxymethyl cellulose method (A. Bezkorovainy and D. G. Doherty, Archives Biochem. Biophys. 96, 491, 1962), and a detailed study of its molecular weight and thyroxine-binding properties was made. Molecular weight was determined by sedimentation-diffusion, sedimentation-viscosity-, and Archibald methods, and was found to be 48,000 at pH 7.0. The weight-average molecular weight increased when pH was decreased below pH 4.0. Thyroxine-binding properties of the M-2 glycoprotein are of considerable interest not only because the system can serve as a model for thyroxine-protein interaction studies, but also because the M-2 glycoprotein bears considerable similarities to the physiological thyroxine carrier of bovine blood. The M-2 glycoprotein did not bind thyroxine in its native form, however, "activation" could be brought about by either heating the protein at 80° for 10 min. or by subjecting it to pH 3 for one hour.

Free energy of binding of thyroxine by the M-2 glycoprotein after "activation" was found to increase linearly with decrease in pH from pH 10.4 to 8.6 and extrapolation to pH 7.4 yielded a $\Delta F = -7.5$ KCal/mole at 27° and -6.8 KCal/mole at 4°C with two binding sites per protein molecule. These binding energies are significant by being higher than those of bovine serum albumin.

C. Bloat in Ruminants.

This work is being done at the National Animal Disease Laboratory at Ames, Iowa, and under cooperative agreements with the California, Maryland, Mississippi, and Wisconsin Agricultural Experiment Stations and with the New York State Veterinary College at Cornell University. In 1961 there was also work done under cooperative agreement with the Minnesota Agricultural Experiment Station. Work is also under way in cooperation with the North Central Regional Project NC-27 on Chemistry and Physiology of Bloat, and through informal cooperation with the Animal Husbandry Research Division.

In 1961 the California Agricultural Experiment Station at Davis, in cooperation with the USDA, collected physiological differences between bloat susceptible and non-susceptible cattle for a third year, and demonstrated an association between bloat susceptibility and salivary secretion rate.

In 1962, at the California Agricultural Experiment Station, a study was made of the relationships between the nature of the diet and rumino-reticular motility. Rumino-reticular motility has been recorded in cattle fed freshly harvested alfalfa tops, Sudan hay, or finely ground, pelleted alfalfa. The frequency and ampliture of mixing and eructation contractions were greater during and following the feeding of alfalfa tops than during and following the feeding of Sudan hay. Pre-feeding motilities were similar. These results suggest that theories which explain bloat prevention by coarse roughages on increased rumino-reticular activity and efficiency of eructation require modification.

Feeding finely ground, pelleted alfalfa hay results in a marked reduction in the ampliture of rumen contractions in comparison with those recorded on long-stem alfalfa hay. This reduction occurs within a few days after the initiation of pellet feeding; motility remains at a relatively low level for as long as 3 months. Recovery of "normal" motility pattern occurs within a week after resuming feeding long hay. A partial explanation of the mild and moderate bloat associated with pelleted and finely ground feeds seems to be a reduction in the strength and efficiency of eructation.

In 1961, in cooperation with the University of Maryland, College Park, a study was made of the pharmacological effects of various sympathomimetic and parasympathomimetic drugs on the eructation mechanism of sheep subjected to various bloat-producing feeding situations. Intramuscular injections of adrenalin and atropine produced failure of eructation and bloat in sheep, goats, and cattle. Oral administration of L-tyrosine also produced bloat, presumably because certain rumen bacteria decarboxylate tyrosine to tyramine, which is toxic to the autonomic nervous system. Since the amino acid, L-tryosine, is a normal component of legume plant protein, a more detailed investigation of the quantitative aspects of this entity in leguminous plant protein has been initiated. Alkaloid fractions of fresh alfalfa and Ladino clover have also given positive bloating responses. The influence of saliva in bloat production is being investigated. Sheep, with their parotid and submaxillary salivary ducts ligated, are being used to determine if sheep with limited salivary secretion, bloat less on alfalfa pasture than untreated sheep. However, thus far no bloat has occurred in either group. A new plastic cannula for gastrointestinal studies has been developed. The cannula appears to be quite adaptable to many physiological studies. Other aspects of this project have involved improving equipment and facilities for a detailed study of the physiology of the eructation mechanism of ruminants.

In 1962, in cooperation with the Agricultural Experiment Station of the University of Maryland, and in informal cooperation with the Animal Husbandry Research Division, ARS, at College Park and Beltsville, Maryland, extracts of alfalfa and Ladino clover were administered to sheep. The bloat response indicated that these legumes contain parasympathomimetic and sympathomimetic compounds in sufficient quantities to be a major factor in natural bloat. Tyrosine, administered orally to sheep, in amounts which could be consumed from green legumes, proved to be sympathomimetic. However, when tyrosine was administered in cottonseed oil, no bloat resulted. In vitro fermentation studies with C^{14} labelled tyrosine indicated that the tyramine complexes with the lipid fraction of the rumen contents.

These results support those in the literature which indicate that the autonomic nervous system is the major system controlling eructation. Results with alkaloid and other extracts of legumes indicate that the para-sympatholytic activity necessary for inhibition of eructation is present in legumes. The indication that the amine derivatives of tyrosine form lipid complexes offers a possible explanation for the usefulness of oils in controlling bloat.

In 1961, in cooperation with the University of Minnesota, an electromagnetic method was adapted and used for the determination of blood flow to the various compartments of the ruminant stomach and to study factors affecting this flow. A better understanding of the factors important to accurate calibration of the instrument has been developed. Results of flow measurements indicated that a blood flow to the forestomach markedly increases during and following feeding. This increased flow appears to be primarily confined to the forestomach and is associated, at least in part, with absorption from reticulorumen.

In 1961, the Mississippi Agricultural Experiment Station, State College, under a cooperative agreement with the USDA, paddocks of hop clover, alfalfa and lespedeza were planted. However, due to unfavorable climatic conditions, insufficiently pure stands were attained for use in producing bloat, therefore, no bloat data was collected for the above-mentioned legumes. Crimson clover was grazed by selected bloater and non-bloater steers. The steers were grazed for 24 days, which allowed 20 days of grazing while the clover was in a bloat-producing stage, and 4 days of grazing in which the steers were not bloating. Rumen samples were taken twice during the grazing period. The first sample was removed after 9 days of grazing and a second sample was removed on the 24th day of the period, thereby giving a rumen sample during the bloat and non-bloat producing stages of growth. The rumen samples are to be analyzed for short chain volatile fatty acids.

In 1962, at the Mississippi Agricultural Experiment Station, rumen samples collected in the spring of 1961 have been analyzed for volatile fatty acids (VFA's). In this study selected bloater and non-bloater steers grazed clover for 1½ hours morning and afternoon. Rumen samples were taken by stomach tube before and after grazing. When the VFA data (milliequivalents of VFA's per 100 ml. rumen fluid) were subjected to statistical analysis the total VFA concentration for bloaters were significantly higher than those for non-bloaters. Significantly more total VFA's were present in samples

taken at 9:30 (after grazing) than those at 7:30 (before grazing). There was a highly significant difference in VFA's present in the rumen fluid. There were significantly more total VFA's in the 9:30 bloater rumen samples than in the 9:30 non-bloater samples.

In 1961, in cooperation with the Wisconsin Agricultural Experiment Station, Madison, efforts were directed toward identifying and characterizing the enzymatic systems of ruminal digestion of leguminous material thought to play a role in the production of increased viscosity of ruminal contents, since it had been demonstrated previously that this is an important factor in producing the physical character of ruminal contents conducive to bubble retention or froth production.

The qualitative determination of pectin methyl esterase (PME) activity was studied by the change in acidity of a pectin solution and by the formation of a pectic gel in the presence of Ca ions and pectin. Alfalfa extracts and a commercial pectinesterase solution always gave a positive reaction in both qualitative tests during the feeding of alfalfa hay to cows - the (centrifuged) ruminal fluid contained no detectable PME activity. The enzyme activity appeared to be associated with the particulate or solid matter.

As a model for estimating the enzymic increases in the viscosity of stomach contents of cows, factors controlling pectin gel formation were investigated, using a commercial pectinesterase. From these studies, potimal amounts of pectin methyl esterase, MaCl, $CaCl_2$ and pectin were suggested which should give a firm gel of Ca pectate in 30-40 minutes.

When fresh alfalfa was extracted with NaCl and added to pectin, the solution gelled within 5 minutes; alfalfa hay extract gelled after 25 minutes. The gelled solutions appeared to liquify enzymically. Higher NaCl concentrated seemed to change the rate of gelling and affect the ultimate equilibrium of the pectin containing system.

Extracts from fresh alfalfa treated with strained, undialyzed ruminal fluid, increased the relative viscosity of pectin to a very high value in 30-40 minutes. Using extracts from centrifuged ruminal fluid, the enzymic increase in the viscosity of pectin was much slower. The heated extracts did not increase the relative viscosity of pectin. The enzymic increase in relative viscosity of pectin also paralleled the protein concentration both for centrifuged and strained ruminal extracts of fresh alfalfa.

High concentrations of NaCl in the extracting buffer appeared to increase the amount of pectin methyl esterase extracted or its enzymic activity. The same NaCl concentrations in the enzymic reaction mixture containing pectin, added $CaCl_2$ and dialyzed fresh alfalfa extracts, caused only a small increase in the relative viscosity of pectin. The higher NaCl concentration is believed to influence the ultimate gel strength and to extend the time required for orientation of pectin molecules to permit cross-bridging in the presence of the optimal $CaCl_2$ concentration.

A crude bacterial pectin degrading enzyme system was prepared for use in identifying the possible pectic substances in frothy, ruminal contents of pasture-bloating cows. The properties of the culture filtrates were studied. Viscometrically the filtrates initially degraded pectic acid 3 to 8 times more rapidly than they did pectin. Their action was like that of an endo-polygalacturonase. Alkyl aryl sulfonate lowered only the initial enzyme activity of these preparations. Dialyzed and lyophilized preparations were retained for further enzymic studies.

When samples of ruminal froth were incubated with various enzymes and inhibitors, a little froth-breaking was observed only in the presence of pectinase, alkyl aryl sulfonate, alpha amylase, Rhozyme H-39, and cellulase. On repeated tests, a higher concentration of cellulase was the most effective preparation against froth stability.

In 1962, at the Wisconsin Station, the feeding of lush, rapidly-growing alfalfa (top 3-4 inches) following 2-3 days on grass or alfalfa hay, produced experimental bloat in cattle. Although the "Before Feeding" sample contained froth, the "After Feeding" samples of frothy digesta were very stable and the froth remained firm and did not break for several hours. Other properties of the bloating process and characteristics of the expressed foamy fluid were observed.

The type of metabolism of the accessible pectin and other associated polymers in the macerated alfalfa plants rather than a large increase of these substances in the rumen cavity may well determine whether the animal bloats. Initial investigations have begun on pectin and pectic substances as the available substrates along with other substances which surround or may limit their release in the rumen when rapidly growing alfalfa is eaten by the cows. Penetration of the waxy surface layers and the cellulose microfibril of these plants may influence the accessibility of these pectin polymers for complete metabolism to products innocuous to bloating.

In continuing studies on factors which influence pectin gel formation by PME, centrifuged rumen fluid produced a firmer gel than water. On the other hand, the rumen precipitate from the centrifuged strained rumen fluid inhibited the pectin gelling when it was added back to the supernatant (centrifuged fluid). Similarly, the properties of pectin methyl esterase prepared in water differed from those reported previously where the enzyme was prepared in the more protective phosphate buffer-sodium chloride medium. This discrepancy will be further investigated.

For ruminal digestion of starchy feed, the role of starch-digesting enzymes and other related substances in the rumen as possible causes of feed-lot bloat, has been initiated. Two experimental systems have been used to evaluate qualitatively and quantitatively the action of starch-digesting enzymes in a commercial source and in rumen fluid from a cow presently feeding on hay.

One method, the starch solution color test, was only useful with soluble starch as the substrate and in these experiments served as a qualitative measure of enzyme activity. The other technique, the agar plate method, measured the quantitative enzyme activity and thus shows great promise for testing inhibitors of these enzymes. This plate method may be used to distinguish between the two fractions of starch. Cornstarch was used with good success when the starch-digesting enzymes were tested on the agar plate method; but this substrate did fail to respond in the solution test. Other reactions are being explored with the agar plate technique.

In 1962, at the National Animal Disease Laboratory at Ames, Iowa, and in previous cooperation with Cornell University at Ithaca, New York, it has been found that eructated gases enter the trachea at pressures approximating those occurring in the esophagus during the expulsive phase of eructation and penetrate deeply into the lungs. Elevation in systemic arterial CO_2 values occur with eructation of this gas. That this increase in arterial CO_2 is not due to the reflex activation of pulmonary arteriovenous shunts is indicated by the fact, among others, that arterial levels greatly exceed the CO_2 content of simultaneously collected venous blood samples. The maximum increase in arterial CO_2 occurs in conjunction with eructation, that is, the arterial samples collected at the level of the carotid artery would have been in the pulmonary circulation at the time of eructation, indicating that there is direct passage of CO_2 into the pulmonary arterial circulation during eructation. This increase in arterial CO_2 is not due to breath-holding or increased intratracheal pressure alone.

That the pulmonary system provides a route of absorption of eructated gas is further substantiated by the finding that various gases, including CO_2, CO, H_2S, and O_2, after being placed in the rumen, are more readily capable of causing changes either in blood-gas levels or in the physiologic activity of the animal when the trachea is patent and capable of receiving these gases during eructation.

The following observations have been made:

1) A nasopharyngeal sphincter closes during eructation and prevents expulsion of gas through the nose.

2) The nasopharyngeal sphincter is formed by the levator veli palatini and oral pharyngeal constrictor muscles.

3) The oral pharyngeal constrictor muscles of the ruminant are involved in the absorption of gas by the blood from the lungs.

4) The glottis remained open during the active phase of eructation, and eructated gas was forced into the trachea.

These observations, together with experiments reported elsewhere on the absorption from the lungs of eructated gases, indicate that a significant amount of eructated gas is forced into the lungs.

Two cows, with ruminal and tracheal fistulas, were used in a series of experiments to determine the depth of penetration of eructated gases into the lungs and the effects of these gases on the flavor of milk. They were also used in experiments designed to compare the lungs to the rumen in effectiveness as absorptive-routes for substances causing off-flavors in milk.

Substances causing off-flavors in milk were detectable in the milk much sooner when given by the lung route than when given by the digestive tract route. For example, detectable off-flavors appeared within 15 minutes after substances were introduced into the lungs, whereas, when substances were introduced into the rumen, it required at least 30 minutes.

It was necessary to incubate macerated onions with rumen ingesta before off-flavors were detected by organoleptic methods in the milk when the lung route method of administration was used. Introduction of macerated onions into the rumen gave rise to a strong off-flavor. The off-flavor appeared sooner when the eructated gases were permitted to enter the lungs than when they were blocked from entering the lungs. Eructated gases also were found to play a role in the transmission of ethyl and amyl acetates from the rumen to the milk.

D. Preparedness for Diagnosis for Foreign Animal Diseases.

In 1961, at the Plum Island Animal Disease Laboratory, studies conducted in swine with rinderpest virus, the suceptibility of this species to the virus was established, and swine have been shown to harbor the virus in bone marrow for as long as 36 days post-inoculation. Complement-fixing antibodies were demonstrated in the serum of swine and in cattle, indicating the usefulness of this test for diagnosis of rinderpest in these species. Beta propiolactone has been found useful for inactivating rinderpest virus in immune serum, and it has been shown to have no effect upon virus-neutralizing or complement-fixing antibodies.

In the course of preparation of materials for diagnosis of Teschen disease, limited observations were permissive in animals. With the swine tested, pigs more than 10 weeks of age were resistant to infection by experimental inoculation.

Competence in diagnosis of vesicular exanthema was achieved during the reporting period and methods were developed for complement-fixation and agar-diffusion tests, using vesicular exanthema virus or serum from convalescent animals.

The hemadsorption test for diagnosis of African swine fever has been applied during the reporting period at the Plum Island Animal Disease Laboratory, and the virus has been serially passaged in tissue cultures.

In 1962, at the Plum Island Animal Disease Laboratory, capabilities for diagnosis of fowl plague, contagious bovine pleuropneumonia, rinderpest, foot-and-mouth disease, Teschen disease, African swine fever, and vesicular exanthema have been obtained. During the reporting period, work has been conducted on rinderpest, African swine fever and foot-and-mouth disease in diagnostic investigations. In work with rinderpest virus, it has been shown that the virus may be transmitted from swine to cattle. However, transmission from infected cattle to swine had not been previously shown. In work conducted at this laboratory, a group of 15 pigs became infected when housed with 2 steers showing signs of rinderpest.

The relationship of distemper, measles and rinderpest has been studied. Monkeys injected with rinderpest virus developed rinderpest antibodies, but not distemper or measles antibodies. Monkeys injected with distemper virus developed distemper and rinderpest antibodies, but not measles antibodies. Puppies infected with rinderpest virus developed both rinderpest and measles antibodies, but not distemper antibodies. However, the puppies apparently were protected against challenge with distemper virus; puppies infected with measles virus developed both measles and rinderpest antibodies. Such puppies were also protected against challenge with distemper virus. Each of the three viruses produced homologous, and in some instances, heterologous antibodies in an alien host. From this work, it may be concluded that after natural or artificial exposure to measles or distemper virus, cattle will not develop antibodies to rinderpest virus that would interfere with the interpretation of serologic tests for rinderpest.

Comparative studies were also conducted with a strain of virus diarrhea and rinderpest viruses. The results of this work suggest that there is no essential serologic or immunologic relationship between the viruses of rinderpest and virus diarrhea insofar as facts are known today.

Rinderpest virus has also been examined by thin sectioning and electron-microscopy techniques. In this work, it has been shown that the effects of infection were visible at 3 hours but development of virus bodies and possible precursor structures occurred later. Rinderpest virus apparently develops within or contiguous to mitochondria as evidenced by small particles of 40-60 mu. which may precede the complete virus of 150-300 mu. in diameter. Virus develops slowly in cells and the final morphological form was visible 7-8 days after infection.

E. Toxicology and Pathology Related to Insecticides

In 1961, at the Toxicological Investigations Laboratory at Kerrville, Texas, in cooperation with the Entomology Research Division, intramuscular and subcutaneous injections of phosphorus-32 labeled Bayer 21/199 as saline suspension, oil suspension/solution, or solution indicated that considerable percentages of the injected materials would be present at the injection site for more than 2 months. Encapsulation of the material would further delay absorption and was observed to occur. Muscle and fat biopsies of a calf, given 1.86 mg/kg of carbon-14 labeled phosphamidon (Ciba) were negative for the material. Biopsies were performed 7 days after dosing. Milk samples from dairy cattle, sprayed with 0.25-0.5 percent equivalents of Shell compound 4294 labeled with carbon 14 or phosphorus-32, indicated apparent residues of 5 to 23 parts per billion. These tiny quantities have not been conclusively identified. Dairy cows were sprayed with phosphorus-32 labeled General Chemical Company compound 4072 at 0.25 percent equivalent. One of the cows received an ADP formulation of xylene and lanolin solution of the compound, the other a regular water emulsion. Apparent residues were present in milk but not identified. The apparent material in milk was reduced by 50% by the ADP formulation.

Shell Compound 4294 (alpha-methyl benzyl 3-(dimethoxyphosphinyloxy) crotonate) was found safe for general livestock use on cattle, sheep, goats, and hogs at the proposed concentration of 0.25 to 0.5 percent in sprays. Toxicity appeared at 0.5 percent in goats; 1.0 percent in sheep, and at higher doses in cattle and hogs.

Ruelene (4-tert-butyl-2-chlorophenyl methyl methyl phosphoramidate) was studied extensively, both as an oral and as a dermal treatment. The maximum non-toxic dosage, administered orally, was 25 mg/kg for young calves; 50 mg/kg for cattle; 100 mg/kg for Angora goats, and 150 mg/kg for sheep. The maximum non-toxic concentration in sprays and dips appeared to be 1.0 percent for young calves and Angora goats; 2.0 percent for sheep and cattle. Cattle were not harmed by 16 treatments with 0.5 percent sprays applied at weekly intervals.

Virginia-Carolina Chemical Co. Compound V C1-13 (O-2,4-dichlorophenyl O,O diethylphosphorothioate). An extensive study of this compound indicated that the maximum safe concentration for use as a spray would be 0.25 percent for calves and goats; 0.5 percent for sheep, and 2.0 percent for cattle, provided the animals are in good flesh. Emaciated sheep were poisoned by 0.5 percent and emaciated Angora goats by 0.25 percent.

Oral toxicity of miscellaneous insecticides. A total of 39 compounds were evaluated for oral toxicity during the year in cattle, sheep, or horses, using only a few animals in each study to give entomologists an indication of the relative toxicity of the compounds prior to their use of them.

Dermal toxicity of miscellaneous insecticides. Four techniques of dermal application were utilized this year in these studies. One method was the customary spraying of animals with 2 to 4 quarts of emulsion or suspension of the compound. A total of 34 compounds were applied in this manner to young dairy calves or cattle.

The second method utilized the same type of emulsions and suspensions, but for particular entomological studies only the hindquarters were treated. Nine compounds were studied in sheep. These materials were promising ones for use in screwworm control.

A third method utilized a procedure now popularly called "pour on." This involves the use of relatively high concentrations with reduced volume simply poured along the dorsal midline of cattle, primarily to obtain cattle grub control by systemic action. A total of 17 compounds were used in these studies.

The fourth method involved the use of high concentrations of insecticide in low volumes of a volatile solvent applied as a mist to the hair. A total of 25 compounds were applied in this manner.

Injectable insecticides. Ten compounds were utilized in these studies by entomologists.

2-pyramidine aldoxime methiodide (2-PAM) was studied as an antidote for Co-Ral poisoning. It did not perform satisfactorily, atropine giving better results even though not entirely satisfactorily.

SKF 525A was not effective as an antidote, again atropine being superior.

Residues of Insecticides in Animal Tissue and Milk:

Sevin. There were no detectable residues of this compound in cattle, sheep, goats, and hogs sprayed 4 times at 4-day intervals with 1.0 percent suspensions. There were no detectable residues in cattle after feeding 50 and 200 parts per million in the total diet for 4 weeks.

Virginia-Carolina Compound V C1-13. Preliminary studies indicate that this compound is stored in the fat of treated animals. Critical studies are being considered for next year.

Oral Toxicity of Herbicides Administered Daily to Sheep: Eleven herbicides were studied in sheep this year. Sheep survived massive dosages for prolonged periods of compounds of 2,4-D; 2,4,5-T; 2-methyl-4-chlorophenoxyacetic acid, and of 2,2-dichloropropionic acid.

Simazine, Atrazine, and dinitrophenol were the most toxic materials studied. The alkamolamine salts of dinitrophenol were much less toxic than the phenol.

In 1962, at the Kerrville, Texas, laboratory, many of the treatments summarized here were administered by entomologists of the Entomology Research Division as a part of their insect control research. ADP personnel observed these animals for evidence of toxicity. Other studies were entirely the responsibility of ADP.

ENT 17,470 (Virginia-Caroline VC 1 - 13) was not toxic to cattle fed 3 mg/kg of the compound in their feed daily for 10 days.

ENT 19,763 (Neguvon, Dipterex, Bayer L 13/59) was toxic for cattle given a single oral dose of 100 mg/kg orally in capsules, but was not toxic at 50 mg/kg, whether the compound was in its technical form or combined in a polymer.

ENT 20,738 (DDVP, Vapona) Horses were not poisoned by doses of 50 or 100 mg/kg of the compound mixed with their feed a single time.

ENT 20,852 (Butonate). Cattle were not harmed by mixing 5 or 10 mg/kg of Butonate with their feed daily for 10 days. Horses given 25 or 50 mg/kg as single doses with a stomach tube showed diarrhea at 25, depression at 50 mg/kg. Mixing Butonate with feed of horses at 10, 25, or 50 mg/kg did not affect them.

ENT 23,708 (Stauffer 1303-E). Spray applications of 0.1 percent and higher poisoned dairy calves 1 to 2 weeks of age. Cattle were not poisoned by 0.05 or 0.1 percent sprays.

ENT 24,650 (Dimethoate, Cygon, American Cyanamid). Dimethoate was not toxic to cattle given 15 mg/kg orally, either as the technical compound or combined in a polymer.

ENT 24,969 (General Chemicals 4072). G.C. 4072 was not toxic to cattle given 2.5 mg/kg mixed with their feed daily for 10 days nor when applied in 250 ml. quantities of 1 percent solution in oil or suspension in water poured along their backs. A horse was unaffected by a single dose of 50 mg/kg of the compound mixed with feed.

ENT 24,988 (DiBrom, California Spray Chemical Corp.). DiBrom had, in previous years, been shown to be too highly irritant to use as a regular cattle spray. Procedures recommended by the company for mist spraying daily with 0.67 percent DiBrom in sugar water severely injured the eyes of the cattle so treated, the effects beginning after 5 daily applications.

ENT 25,540 (Bayer 29,493). Cattle were not poisoned by daily doses in their feed of 2.5 mg/kg of Bayer 29,493. Single intramuscular injections of 2.5 mg/kg were not toxic to cattle but doses of 5 mg/kg poisoned 3 of 4 cattle so treated.

ENT 25,561 (Grace-Ack 58-26). Cattle were poisoned by single oral doses of 25 mg/kg of ENT 25,561 administered in capsules.

ENT 25,602 (Ruelene). Horses were not poisoned by single doses of 25 or 50 mg/kg of Ruelene mixed with their feed.

ENT 25,644 (American Cyanamid 38,023). Cattle were not poisoned by feeding them 10 mg/kg of 25,644 daily for 10 days, nor by single intramuscular doses of 10, 15 or 20 mg/kg.

Sheep were unharmed by 25,50 or 100 mg/kg intramuscular injections. Sheep were poisoned by oral drenching at 100 mg/kg. A horse was unharmed by a single dose of 50 mg/kg administered with a stomach tube.

Ent 25,702 (Bayer 39,193). Sprays of 0.5 percent concentration were toxic to cattle. Cattle were not poisoned by oral doses of 50 mg/kg administered in capsules.

ENT 25,705 Stauffer R-1504). Cattle were not poisoned by sprays containing 0.5 percent of 25,705 but were poisoned by pouring 250 ml. quantities of 2 percent oil solutions or water suspensions along their backs. Single oral doses of 10 mg/kg were tolerated but 25 mg/kg poisoned cattle. Feeding cattle 2.5 mg/kg daily for 10 days did not produce poisoning.

ENT 25,712 (Bayer 37,289). Cattle given single oral doses in capsules at 10 mg/kg were unaffected.

ENT 25,714 (Bayer 38,333). Sprays of 0.25 percent were toxic to cattle. Single oral doses of 10 mg/kg in capsules poisoned 3 cattle so treated.

ENT 25,725 (Bayer 42,600). Sprays of 0.5 percent and oral doses of 25 mg/kg in capsules poisoned all cattle so treated.

ENT 25,749 (Shell 7394). Dairy calves 1 to 2 weeks of age were not affected by oral doses of 15 mg/kg but were poisoned by doses of 25 and 50 mg/kg.

ENT 25,763 (Hercules 7522H). Dairy calves 1 to 2 weeks of age were poisoned by single oral doses of 10 mg/kg and higher. Cattle were not poisoned by single oral doses of 100 mg/kg administered in capsules.

ENT 25,766 (Dow Zectran). Angora goats were not poisoned by 0.25 percent concentrations of Zectran.

ENT 25,769 (Stauffer N-2310). Cattle were not poisoned by single oral doses of 10 mg/kg administered in capsules.

ENT 25,780 (Hooker 1422). Sprays containing 0.25 and 0.5 percent of 25,780 were not toxic to cattle.

ENT 25,795 (Stauffer N-2599). Dairy calves 1 to 2 weeks of age were poisoned by oral doses of 12 mg/kg but not 6 mg/kg. Yearling cattle were poisoned by single oral doses of 20 mg/kg administered in capsules.

ENT 25,797 (Stauffer N-3047). Dairy calves were poisoned by single oral doses of 15 mg/kg and higher. Doses of 8 mg/kg were tolerated. Yearling cattle were not poisoned by 15 mg/kg.

ENT 25,798 (Stauffer N-3054). Dairy calves were poisoned by oral doses of 8 mg/kg and higher and were killed by such doses of 30 mg/kg. Cattle were poisoned by single oral doses of 10 mg/kg administered in capsules.

ENT 25,799 (Stauffer N-3055). Dairy calves were poisoned by single oral doses of 10 mg/kg and higher and were killed by doses of 20 mg/kg and higher. Yearling cattle were poisoned by single oral doses of 10 mg/kg administered in capsules.

ENT 25,801 (Stauffer R-3422). Dairy calves were not poisoned by oral doses of 15 mg/kg but were poisoned at 25 mg/kg. Yearling cattle were poisoned by single oral doses of 20 mg/kg administered in capsules.

ENT 25,810 (Hercules 9699). Dairy calves were not poisoned by single oral doses of 8 mg/kg but were poisoned by doses of 15 mg/kg and higher. Yearling cattle were not poisoned by single oral doses of 13.4 mg/kg administered in capsules.

ENT 26,613 (Rhodia 9895). Dairy calves were not affected by sprays containing as much as 3 percent of this compound. Yearling cattle were unaffected by 2 percent sprays or single oral doses of 100 mg/kg. Feeding 15 mg/kg daily to cattle for 10 days produced poisoning.

Compounds used to induce sexual sterility in insects appear to have a definite cumulative effect upon sheep. Poisoned animals react rather suddenly, even in long-term studies. It is worthy of note that of the two compounds studied, aphoxide, reported to be the more effective chemosterilant, is also by far the more toxic. Such compounds will need to be used most carefully to avoid damage to desirable forms of life.

ENT 26,316 (Apholate). Sheep were killed by single intramuscular injections of 5 mg/kg and by 11 weekly injections, each 0.5 mg/kg. Sheep were killed by single oral doses of 50 mg/kg and by 11 doses of 25 mg/kg administered at weekly intervals. Weekly doses of 5 or 12.5 mg/kg were not toxic after 20 doses, at which time the test was terminated. When administered daily to sheep, deaths followed 11 daily doses of 20 mg/kg; 19 and 22 daily doses of 10 mg/kg; 19 and 25 daily doses of 5 mg/kg and 101 and 128 daily doses of 2 mg/kg. Sheep receiving 1 mg/kg daily have survived 168 daily doses and will be continued in test.

ENT 24,915 (Aphoxide). This compound caused deaths in sheep after 7 and 9 daily doses of 5 mg/kg and after 6 daily doses of 2 mg/kg.

Antidotes for poisoning. Atropine was used extensively throughout the year to save most of the animals poisoned in our own studies and in many of those conducted by the Entomology Research Division. Atropine is still by far the most satisfactory antidote for organic phosphorus and carbamate compound induced poisoning. BAL (dimercaptopropanol) was not useful as an antidote for poisoning by organophosphorus compounds.

Residues of insecticides in meat and milk. These studies were conducted cooperatively with the Entomology Research Division and the companies interested in the compounds.

ENT 17,470 (Virginia Carolina VC 1-13). This study involved sheep, goats and cattle sprayed a single time with 0.5 percent suspensions. Substantial residues appeared in the fats and in smaller amounts in other tissues. The residues declined, reaching extinction at 6 to 8 weeks after treatment.

ENT 19,507 (Diazinon). This study involves only cattle that are sprayed weekly with 0.05 and 1.1 percent suspensions of diazinon. A total of 16 applications are to be made, the final one coinciding with the end of this reporting year. Omental fat samples have been taken and are being analyzed. The test will be reported in full in FY 1963.

Herbicides:

Banvel D (Velsicol). Sheep were not poisoned by doses as high as 1000 mg/kg.

Bandane (Velsicol). Sheep were not poisoned by 200 mg/kg of Bandane but a sheep given 1000 mg/kg was killed. Signs of poisoning were mostly those of central nervous system origin.

Studies were initiated in the area of fungicides that might be used in pasture and field treatment. These were started June 4, 1962.

Captan was lethal for a sheep given a single oral dose of 500 mg/kg. One sheep given 100 mg/kg daily showed signs of poisoning after 3 doses. There is some indication that Captan may have an anticoagulant effect upon the blood.

A Jersey cow was sprayed with carbon-14 labeled Ciodrin (Shell Compound 4294) to obtain data on the excretion of the compound in milk. The conditions imposed by the milk study required the cow to be stanchioned most of the time, giving us an opportunity to study residues of the compound on the hair, unaffected by the normal licking and tail-switching activities of the cow. Under these conditions the residue of Ciodrin on the hair did not decline appreciably in 2 weeks, but the distribution between tight and shedding hairs changed from a proportion of 40/60 to 10/85 in one week, indicating that licking and tail switching would have accounted for removal of more than 80 percent of the applied material had they been permitted. Ciodrin did not appear to migrate along the hair after the spray had dried.

F. Biochemical Effects of Agricultural Chemicals

In 1961 at the Kerrville, Texas, Station, studies were made on sheep penned, then pastured, of glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), malic dehydrogenase (MOH), and aldolase in blood sera. Approximately a two-fold increase of serum malic dehydrogenase occurred in sheep after 3 weeks on pasture. No other differences were noted. During the study several individuals showed sudden increases in activity of GOT and aldolase, without clinical signs. Biopsies of liver revealed infiltration with polymorphonuclear leucocytes and moderate infiltration with fibroblasts. There were no remarkable changes in prothrombin time, chlorides, calcium phosphorus, creatinine, non-protein nitrogen, urea nitrogen or uric acid in bloods of sheep receiving representative herbicides for prolonged periods of time.

In 1962 at the Kerrville Station, in a cooperative study with researchers of the Wm. Cooper and Nephews Company, Angora goats and crossbred sheep were given Coopex (phenothiazine plus coroxon) orally, then sprayed immediately or two weeks later with organic phosphorus compounds. A marked potentiation of toxic effects and cholinesterase inhibition was noted in the goats sprayed immediately after drenching when the sprays contained normal concentrations of Delnav or VC 1-13. These effects were not so marked when the sprays contained malathion or Bayer 21/199. The potentiation did not appear when the spraying was delayed two weeks. Sheep drenched, then sprayed on the same day, as were the goats, did not respond to a greater degree than when Coopex was given alone.

The rather remarkable potentiation of effects in goats as opposed to those in sheep indicate that, in the goat, administration of phenothiazine must interfere with the detoxification or metabolism of organic phosphorus compounds present in the body at the same time. There were 3,641 determinations made of the cholinesterase activities in as many samples of blood from experimental animals. There were 119 chemical determinations of hemoglobin and 234 of serum bilirubin. In addition, 119 determinations were made of prothrombin time, 110 of packed cell volume in blood. The results of these determinations, for the sake of understanding and brevity, are reported with the main studies. In addition, as a part of the Kerrville Laboratories safety program, 51 determinations were made of erythrocyte and a like number of plasma cholinesterase activities in the blood samples of the Station's personnel.

In cooperation with the Stephen F. Austin State College at Nacogdoches, Texas, various types of equipment have been investigated to determine their possible use in constructing and calibrating an ultrasonic particle size spectrometer. Techniques for calibrating such a spectrometer have been worked out. The major effort has been directed toward a theoretical and experimental investigation of the scattering of plane acoustic waves by rigid spheres. It has been found that a measurement of the scattering pattern leads to a determination of the size of the sphere. Immediate plans are to continue this investigation using an array of solid spheres as well as liquid drops.

G. Detoxication Mechanism in Cattle and Sheep.

In 1962, at Kerrville, Texas, efforts were concentrated toward successfully developing an analytical method, with appropriate "clean-up" procedures, successfully developing paper chromatographic and electrophoretic methods for identification of 2,4-D, proving both the above methods by use of carbon-14 labeled 2,4-D, and studying the absorption, excretion and tissue residues of 2,4-D in a sheep given a single oral dose of 4 mg/kg.

In the sheep given the 4 mg/kg dosage of 2,4-D maximum concentrations of the compound in blood were reached in 2 hours. At 8½ hours after dosing 50% of the administered dose had been recovered in the urine and 90% had been recovered 28 hours after dosing. In the urine 2,4-D was the only radioactive material found. The sheep was destroyed 4 days after treatment. Tissues obtained at necropsy were analyzed, yielding an average apparent residue of 2.6 parts per billion.

The highest tissue residue occurred in the thyroid gland, amounting to only 0.56 part per million, but this was far more than occurred in any of the other tissues. This finding lead us to wonder whether or not the 2,4-D had been mistaken in the body for a precursor of thyroxine such as mono- or diiodo-tyrosine.

H. Cytological Responses to Antiparasitic and Other Agricultural Chemicals.

In 1962, at Kerrville, Texas, it was found that Apholate (ENT 26,316) administered to sheep in small daily doses regularly produced marked decreases of the white cells, particularly the lymphocytes and the polymorphonuclear leukocytes. Marked decreases in blood platelet numbers were noted. The red blood cells, in number, volume and hemoglobin content were not significantly affected. Serum bilirubin and serum prothrombin time were not appreciably affected. Necropsy findings usually involved the circulatory system. All dosages in excess of 1 mg/kg daily have killed the sheep so treated.

Histological examinations, gross and microscopic, of the tissues of sheep poisoned by several organic herbicides, show that the primary effect of those materials is upon the kidney, followed closely by the lungs. Other tissues may be involved with hemorrhages and other types of generalized changes.

During this reporting period 87 sets of tissues were obtained for processing. A total of 3,648 routine hematoxylin-eosin stained sections were prepared. Special staining was done for 310 slides, including periodic acid - Schiff, Gomori, Gridley fungus, Giemsa, Gram's, and amyloid and fat stains. Wright's stain was used for 140 blood smears.

I. Poisoning by Plants.

In 1961, at the Logan, Utah, poisonous plant field station, studies were made to determine the cause of congenital cyclopia-type malformation in lambs for the sixth consecutive year in cooperation with the U. S. Plant, Soil and Nutrition Laboratory of the Soil and Water Conservation Division, and the Utah Agric. Exper. Station. Veratrum californicum, commonly called False hellebore, Wild Corn, and Skunk cabbage, fed to 51 bred ewes for 30 days, caused a congenital cyclopia-type malformation in the lambs of 17% of the ewes, and fetal deaths in 29 percent. This is the first time a poisonous plant has ever been experimentally proven to be associated with a congenital deformity in animals. The Veratrum californicum plants, collected at different locations, showed a marked variation in their ability to cause toxic symptoms in ewes and congenital deformities in lambs. The commercial alkaloids of Veratrum californicum caused toxic symptoms similar to the veratrum plants when fed to sheep. Veratrum californicum is definitely a poisonous plant for sheep, causing characteristic toxic symptoms and pathological changes in certain organs. A congenital cyclopia-type malformation has been occurring for the past 60 to 70 years in lambs born from ewes bred in August and early September on certain areas of the summer ranges in the Boise, Sawtooth, and Challis National forests. This deformity has always been looked upon by the livestock men, and many individuals in scientific research, as a hereditary disease. The number of affected lambs has ranged from 1 to 25 percent in individual herds of 3,000 to 10,000 ewes each. The congenital anomaly is related to a condition of prolonged gestation in ewes bearing the affected lambs. Such ewes are sold as culls or die during parturition and results in a two-fold loss which is estimated to range from $15,000 to $100,000 annually for the sheepmen in southeastern Idaho.

The investigations of locoweed poisoning in sheep were indicated in the previous work reported in ADP a7-7 annual report 1960, to cause abortion and congenital deformities in sheep. Loco poisoning in ewes has often been suspected by the sheepmen as a possible cause of increased number of dry ewes and congenital deformities in lambs when poisoning occurs during the gestation period. In 1960, 9 of 22 ewes aborted. One aborted fetus and 3 full-term lambs had from slightly - to - marked twisted front legs. In further investigation of this problem, twin full-term lambs were born from a ewe fed Oxytropis lambertii (locoweed), with congenital deformities of front legs. One lamb showed contracted pastern joints of both front legs, and the other twin had contraction of the pastern joint in only the right front leg. Both animals walked on the distal end of the metacarpus bone. Three rams fed locoweed daily showed marked decrease in physical vigor and decrease in semen quality, which was reflected in the morphology of the sperm collected by electrical ejaculation.

Fourteen ewes were maintained on a locoweed-free diet after suffering from loco poisoning and abortion. The ewes were re-bred the following year. All conceived at the first breeding period and gave birth to normal full-term lambs. This indicated that there is little or no danger of a carry-over of reproductive disorders following the recovery of locoweed poisoning in ewes. One 2-year old Holstein cow was fed locoweed (Astragalus lentiginosus) from

the 41st to her 100th day gestation. She gave birth to a full-term normal calf, as compared to 2 heifers fed the previous year and aborted fetuses with twisted front legs.

Studies to determine the etiology of a congenital deformity in calves, commonly called "crooked calf disease," have been continued for the 6th consecutive year in cooperation with the U. S. Plant, Soil and Nutrition Laboratory of the Soil and Water Conservation Division. The disease has been found to occur in Idaho, Washington, Montana, Wyoming, Oregon, California, Nevada, and Utah. It is likely that the disease is occurring in many other States, but not recognized because the livestock men have always considered the anomaly to be of hereditary origin and kill the affected calves soon after birth. The disease has caused an estimated loss of $500,000 to $1,000,000 annually to the livestock industry.

The disease has been known to occur ever since records were kept on range cattle. The Animal Disease and Parasite Research Division researchers were the first to start a detailed scientific study to determine the etiology of this deformity. A congenital deformed calf, typical "crooked calf disease," was reproduced for the second time by feeding a Holstein heifer Lupinus sericeus and lead daily from the 56th to the 115th days of the gestation period, but 9 heifers, fed lupine without lead, all gave birth to normal calves. Lupinus argenteus, fed to 5 ewes twice daily for 45 days starting on day of breeding, caused marked general body weakness and slight liver dysfunction. All ewes conceived on first breeding, and gave birth to full-term live normal lambs.

The halogeton plant (Halogeton glomeratus) has spread from its original site, first identified in the United States in 1934 at Wells, Nevada, to all eleven western States. The plant is very difficult to eradicate once it becomes established due to its prolific ability to produce large numbers of resistant and dormant seeds.

Investigations from this Station have shown that sheep losses from halogeton poisoning can be prevented by supplementing the feed with dicalcium phosphate. The supplement has been effective when added to grain or alfalfa pellets at the 5% level and fed 1/4 lb. daily when animals are grazing in the halogeton-infested areas.

The use of dicalcium phosphate as a feed supplement has saved the sheep industry many thousands of dollars by preventing losses from halogeton poisoning. Dicalcium phosphate is the only mineral supplement which will provide 100 percent protection. Bone meal, a mineral supplement recommended for livestock for many years, makes the animals more susceptible to halogeton poisoning.

In 1962 at Logan, Utah, the study on the cyclopia-type malformation in lambs, which has been shown to be caused by the maternal ingestion of Veratrum californicum, has been continued in cooperation with the USDA Soil, Plant and Nutrition Laboratory, Ithaca, New York. After it was discovered that

Veratrum californicum was a poisonous range plant and when ingested by ewes early in the gestation period would cause congenital deformities in the lambs, the sheepmen who kept their animals away from the plant during the breeding season have eliminated this disease condition. These men reported that it was the first time since they had been in the sheep business that they did not suffer losses from this deformity. It is estimated that the men who complied with the recommendations to keep their sheep away from the Veratrum californicum plant during the breeding season saved from $1,000 to $5,000 each in their 1962 lamb crop.

Various etiological agents are known to cause congenital malformations. So as to eliminate the unknown variables of high altitude (anoxia), transportation and the drying of plants, the main experiment was conducted on the range in Muldoon Canyon, Idaho. The experiment was specifically designed to associate the incidence of congenital deformities, severity of deformities, and the specific time of insult on the developing embryo with this disease condition.

Based on this year's work additional and useful information regarding this disease was obtained. Malformed lambs were born to ewes that were fed Veratrum californicum for 15, 20 and 30 days after the day of breeding. No malformed lambs were born from ewes fed the plant 5 and 10 days from the day of breeding. The incidence and severity of the malformations could not be associated with the degree of clinical toxic symptoms of this disease in the ewe. Embryonic and fetal deaths appear to be associated with this disease process; however, more controlled data and information is needed regarding this one aspect of the disease. The commercial veratrum alkaloid did not induce any malformed lambs, but did produce typical symptoms in the ewes.

Some of the experimental plant (Veratrum californicum) was harvested and hauled to Logan, Utah. After being dried and chopped, it was stored for 3 months and fed to ewes. Malformed lambs were produced under such procedures, but the incidence of malformations was lower than with the green (undried) plant. More information is needed regarding the degree of toxicity associated with the various stages of maturity of the plant, various locations where grown, and proper method of storage to maintain toxicity.

The substance in the plant responsible for causing the deformity is unknown. Further studies have been designed to make this determination. Early investigations have shown that the veratrum plant contains various alkaloids. A chemical analysis was made of Veratrum californicum by Physics and Chemical Investigations of the National Animal Disease Laboratory and showed the specific species to contain alkaloids.

Commercial veratrum alkaloids of cevadine, V. alpha and V. sulfate were obtained to determine their effect on foetus of ewes and chick embryos. Cevadine fed to ewes at 5 mg. per kilogram of body weight caused clinical symptoms identical to poisoning by Veratrum californicum, but failed to cause congenital malformations in lambs when fed at 3 mgs. per kilogram of body weight for the first 15 days of gestation. Cevadine, V. alpha and V. sulfate caused marked retardation in growth and development of chick embryos. Inoculations were made at various levels into the egg yolk from 24 hours before start of incubation to 7 days after incubation.

It is difficult to evaluate, at this time, the relationship of lupine or lupine plus lead to the etiology of "crooked calf disease" until more experimental data is available. As epidemiology studies are made and experimental feeding trials carried out, they seem to indicate, more each year, that the causative agent for "crooked calf disease" is of plant origin.

Clawson and Huffman, in 1937, showed that the ingestion of Tetradymia glabrata by range sheep would cause "Bighead disease." The results in experimental feeding trials have been very erratic. The plant was poisonous and in toxic doses would commonly cause death with severe liver and kidney damage. The swelling of the head would not always be produced even though the animals were in direct sunshine. This problem has always been of much concern to us in the study of the effects of poisonous plants on livestock.

There may be another plant the sheep must eat, either before or along with Tetradymia glabrata, in order to make the animal sensitive to the sun in order to cause marked edema and swelling of the head. The purpose of this experiment was to make a preliminary study to determine if further investigations would be justified another year.

The "Bighead" disease was unable to be reproduced by feeding Tetradymia plus alfalfa hay, Tetradymia plus green grass, Tetradymia alone, or Tetradymia plus big sage. Two animals died with severe liver and kidney damage, and all others were sick and off feed for three days following the feeding trial.

Investigations to determine the relationship of locoweed poisoning to congenital deformities in cattle and sheep have been continued. Previous studies reported in annual report of ADP a7-7, 1960 and '61, showed congenital malformation in legs of lambs produced by maternal feeding of Oxytropis lamberti, Astragalus pubentissimus and A. lentiginosus.

In the years when there is considerable rainfall on the winter ranges during September, October, and November, with moderate temperatures and no snow, loco plants grow very profusely. As the animals come from the summer ranges to the winter ranges in late October and early November, the locoweed have all made new growth and are from 4 to 10 inches in height. Once the animals start eating the plants they tend to ignore other range forage and look only for the locoweeds. In the years when such conditions occur, they are always followed by serious livestock losses from loco poisoning. Bands of sheep suffering from loco poisoning have been known to have as much as 80 percent abortions with a 15 percent death loss in the ewes.

There is no known prevention or treatment for the disease at this time. The plants are so widespread that it is impractical to attempt control of the plants by herbicides. Further studies are necessary to determine the effect of possible alleviators to prevent the poisoning in the animals and new methods for eradicating the plants.

In 1962, under a PL 480 grant to the Instituto Biologico, Sao Paulo, Brazil, Mascagnia pubiflora was found to be abundant, the leaves were toxic, and the plant was causing extensive losses in cattle. The lethal dose was found to be about 4 percent of the body weight. The symptoms and lesions were determined and guinea pigs and rabbits were also found susceptible to its toxicity. Studies are also under way on the isolation and chemical identification of toxic principles of Palicourea marogravii, and Sessea brasiliensis.

J. Toxicity of Herbicides and Herbicide-Treated Plants for Domestic Animals.

In 1962, at Logan, Utah, in cooperation with the Crops Research Division of ARS, and the Utah Agricultural Experiment Station, the following report is submitted.

Since Veratrum californicum has been found to be a poisonous plant for sheep and will cause a congenital cyclopia-type malformation in lambs, marked interest has been stimulated in the sheepmen to know how herbicides may affect its toxicity for sheep. Four 1/100 acre plots of Veratrum californicum plants were laid out in Logan Canyon. One was sprayed at a rate of 2 quarts per acre of 2-4D and one 4 quarts per acre. Two other plots were sprayed with 2-4-5T. One plot was at a rate of 3/4 lb. per acre and the other 1.5 lbs. 2-4-5T per acre. The plants varied from 1 to 2 feet in height, all leaves were dark green color and plants were making rapid growth at the time they were sprayed. Seven days later the leaves of the treated plants were starting to show a rust-brown coloration and growth rate seemed to be retarded compared to the plants on the outside of the plots.

One and three-fourths lb. of the untreated green veratrum plant was required to produce mild toxic symptoms in sheep. Three weeks after the plants were sprayed with 2-4D, 2 1/2 lbs. of the plant failed to cause symptoms while 2 1/2 lbs. of the plants sprayed with 2-4-5T caused mild toxic symptoms.

A herbicide that will kill the veratrum plants without destroying other types of forage or reduce its toxicity to prevent poisoning and congenital malformations in lambs is urgently needed by the sheepmen. Such a herbicide would save time, extensive work and many lambs and ewes that are now being lost from ingestion of the plant.

From this preliminary investigation, 2-4D applied as a spray at a rate as low as 2 quarts per acre was effective in reducing the toxicity of the veratrum plant to sheep, while 2-4-5T seemed to have very little effect at the highest rate of application (1.5 lbs. per acre).

This past year some of the sheepmen have preceded their sheep to the summer range areas and cut the veratrum plants down by hand. If 2-4D can be used as effective on the ranges as it was on the experimental plots to kill the veratrum and prevent toxicity it will save considerable time, expense and labor.

K. Alleviators and Diagnostic Tests for Plant Poisoning.

In 1961, at the Logan, Utah Field Station, dicalcium phosphate was shown to be very effective in the prevention of halogeton poisoning in sheep.

In 1962, in further studies with dicalcium phosphate in relation to halogeton poisoning, it has also been found very effective in the prevention of "dirt eating" in lambs and cattle and stiffness in range lambs at 2 to 4 months of age.

The sheepmen who have followed the recommendations of supplementing their animals free choice the year around with a mixture of 50 lbs. ground salt and 50 lbs. of 18% dicalcium phosphate have suffered only very slight losses from halogeton poisoning and no losses in lambs from "dirt eating" or stiffness of lambs on the range. In determining the effectiveness of dicalcium phosphate as an alleviator to cattle and sheep in the prevention of halogeton poisoning, "dirt eating" in lambs and stiffness in range lambs has saved the livestockmen many thousands of dollars. The saving comes not only in the prevention of animal losses, but in the cost of the mineral. Commercial minerals are sold from $6.00 to $20.00 per hundred while salt and dicalcium phosphate mixture may be obtained from $5.00 to $6.00 per hundred with uncomparable results on the animals.

Blood tests of sheep fed halogeton show an increase in the potassium and sodium ions and urea nitrogen. Animals fed halogeton plus dicalcium phosphate showed no marked increases of potassium, sodium and urea nitrogen. The calcium and phosphorus ions in dicalcium phosphate seem to be more readily metabolized than from other mineral supplements and their alleviating effect may be found effective in many metabolic disturbances.

L. The Susceptibility of Wild Animals to Foot-and-Mouth Disease.

In 1961, at the Plum Island Animal Disease Laboratory, the susceptibility of horses, cats, rats, dogs, monkeys, frogs, and steers to foot-and-mouth disease virus (FMDV) and its ribonucleic acid (RNA) were compared. They were injected intramuscularly or on the tongue with tissue-cultured FMDV, type A, strain 119, using a dosage proportionate to the weights of the animals. Blood was collected 24 hours after injection and tested for demonstrable virus in steers, mice and tissue cultures. Another group of animals of the same species was treated similarly, using RNA derived from FMDV as the inoculum. Virus was demonstrated in the blood of cats, rats, and steers 24 hours after inoculation with intact FMDV, and after inoculation with RNA. Monkeys and frogs developed a viremia only after inoculation with intact virus. Horses and dogs failed to develop a viremia following inoculation with intact virus or its RNA. Comparable results were obtained in the three test media used, i.e., steers, mice and tissue cultures. A few muskrats trapped on Plum Island were inoculated in the metatarsal pads with FMDV, guinea pig strains A-GB or C-GC. Blood was collected from the animals 24, 48, and 72 hours after inoculation and tested in guinea pigs. The $ID_{50/ml}$

titers of FMDV in the blood of the muskrats ranged from 3.0 to 3.5 at 24 hours, 2.5 to 4.5 at 48 hours, and 2.8 to 3.8 at 72 hours after inoculation. The titers obtained and the duration of the viremia indicated that the virus multiplied in the muskrats.

In 1962, workers at Plum Island report that previous experiments indicated that monkeys, cats, rats, muskrats and frogs develop viremia after inoculation with FMDV. Of these animals, only muskrats had clinically recognizable lesions. Horses and dogs did not develop viremia after inoculation. When RNA of FMDV was used, only cats and rats had viremia; monkeys, horses, dogs and frogs did not.

Nine chinchillas (chinchilla langiera) were infected by intradermal inoculation of both metatarsal pads with 0.1 ml of 10% suspension of FMDV A-119 representing the 18th guinea pig passage. Within 30 hours, vesicles developed in all inoculated sites. Secondary lesions developed from the 2nd to 9th day postinoculation, and 5 chinchillas died during this time. Blood samples for serologic examination were taken from all animals immediately before inoculation and from surviving animals 14 and 22 days postinoculation. Virus-neutralization tests in suckling mice were conducted using FMDV A-119 and FMDV O-39 as antigens. Neutralizing antibodies against A-119, but not O-39, were present in the sera of the 4 surviving chinchillas 14 and 22 days post-inoculation.

M. Environmental Stress as a Contributory Factor in Animal Diseases.

In 1962, at Pulawy, Poland, under a PL 480 grant equivalent to $76,187, (E21-ADP-7), basic physiological determinations of the normal values of the basic elements in the blood and tissues of poultry have been determined. Preliminary work on the stress influence of adrenocorticotrophin, Pasteurella multocida, and transportation have also been completed. These preliminaries were necessary before engaging in the experiment proper.

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AREA NO. 8 - FOOT-AND-MOUTH DISEASE AND OTHER EXOTIC DISEASES OF CATTLE

Problem. Responsibility for protection of the Nation's livestock industry against diseases, including those of foreign origin, was delegated to the USDA in 1884. Thereafter, contagious bovine pleuropneumonia eventually was eradicated from the United States, thus reopening European markets for practicable, scientifically justified barriers against introduction of such dangerous exotic diseases as foot-and-mouth disease and rinderpest. The Plum Island Animal Disease Laboratory was established for scientific support of measures for protection against these and other foreign diseases of animals, following the direct threats of spread of foot-and-mouth disease from Mexico and Canada (1946-1954). Foot-and-mouth disease, which is capable of reducing over-all productivity by 25 percent in areas where it becomes established, persists in most major livestock producing countries, except Central and North America, Australia, and New Zealand. Rinderpest continues to be a serious disease problem in Africa and Asia; it is capable of killing 90 percent or more of the cattle that are exposed to it. Other diseases, such as contagious bovine pleuropneumonia, Rift Valley fever, and East Coast fever, continue to exact severe tolls in other parts of the world. Possibilities of entry of these diseases into the United States continue, despite all precautions, primarily because of the progressively increasing scope, speed, and extent of modern international transportation. The purposes of the Plum Island Laboratory are development of basic information applicable to protection of the Nation's livestock from foreign animal diseases; development and maintenance of competence in diagnosis of these diseases; and fundamental research on the biological, chemical, and physical properties of the infectious agents that may be useful in prevention, control, and eradication of these diseases.

USDA PROGRAM

The Department has a continuing long-term program involving veterinarians, biochemists, biophysicists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. All of this research is being conducted on the following diseases at the Plum Island Animal Disease Laboratory, Greenport, Long Island, New York, except for supplemental field studies on vaccines in the Netherlands.

The Federal scientific effort devoted to research in this area, conducted solely at the Plum Island Animal Disease Laboratory, totals 33.3 professional man-years. This effort is divided among sub-headings as follows:

Pathological investigations of foot-and-mouth disease in cattle 1.0

Fluorescent antibody techniques 1.0

Diagnostic investigations 3.0

Susceptibility of cell lines 0.5

Production and maintenance of standardized reference stock of virus and homologous antisera 2.3

Carrier state in convalescent animals 0.5

Parasites in transmission of foot-and-mouth disease 0.5

Foot-and-mouth disease vaccines 4.0

Antigenic variations of foot-and-mouth disease viruses 1.0

Production of foot-and-mouth disease antibody in vitro 0.5

Immune response to various types and subtypes of foot-and-mouth disease virus 1.5

Quantity production of foot-and-mouth disease virus 2.0

Microcinematography of infected cells 0.5

Pure stable lines of culture cells 0.5

Purification of foot-and-mouth disease virus 2.0

Chemical and physical characterization of foot-and-mouth disease virus 1.0

Interaction between foot-and-mouth disease virus and host cells 1.0

Genetic biochemistry of foot-and-mouth disease virus 1.0

Effects of chemical and physical environments of foot-and-mouth disease virus 1.0

Preservation of foot-and-mouth disease virus 1.0

Rinderpest 2.5

Transmission of foot-and-mouth disease virus in semen 1.5

Survival of foot-and-mouth disease in meat and meat products 2.0

Susceptibility of wild species to foot-and-mouth disease 0.5

Adaptation of foot-and-mouth disease virus to poultry and embryonating chicken eggs 1.0

Public Law 480 funds equivalent to $11,572.21 have been made available to the Turkish Ministry of Agriculture for a 2-year study of tissue culture of indigenous strains of foot-and-mouth disease virus and experimental field vaccination.

$78,594 have been allotted to the Biological Institute, Sao Paulo, Brazil, for a 5-year study of tissue culture of indigenous strains of foot-and-mouth disease virus and experimental field vaccination.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

Experimentation with the virus of foot-and-mouth disease in the United States essentially is prohibited by law, except at the Plum Island Animal Disease Laboratory. Experimentations with the causative agents of other communicable, foreign, or exotic diseases of cattle in the United States is similarly prohibited generally by federal regulations. Consequently, the State Experiment Stations are not working with diseases in this category.

Insofar as is known, foot-and-mouth disease is the only one of the foreign diseases of cattle in which American industry has manifested notable interest. Although experimentation with foot-and-mouth disease is prohibited in the United States, except at Plum Island, at least two U. S. biological firms are known to have initiated vaccine production programs in South America, with plans for limited corollary research. It is estimated that no more than about 5 professional man-years are so engaged.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Pathological investigations of foot-and-mouth disease in cattle.

In 1961, studies on the frequency of atypical (non-vesicular) lesions of foot-and-mouth disease in cattle were temporarily discontinued due to emphasis on other lines of cytological investigation. Spontaneous occurrence of so-called metastatic calcification syndrome among guinea pigs used for FMDV experimentation necessitated study of this disease on several occasions. Nephritis was found consistently in affected animals, but cytologic study and volumetric determinations of the parathyroid glands failed to show evidence of parathyroid hyperactivity secondary to renal damage. It was concluded that the calcification syndrome is of dietary, rather than renal origin, and that the scondary kidney lesions probably result from excretion of excess phosphates of dietary origin.

In 1962 work on this project was limited to routine service work in histopathological diagnosis performed in connection with work in other projects. In addition, a large collection of kodachrome slides (256) and prints illustrating animals affected with exotic diseases was selected and furnished to ADE and the Economic Research Service.

B. Fluorescent antibody techniques.

In 1961, two techniques were developed which may be used for demonstrating, localizing and identifying FMDV in tissues. In the indirect technique, the system consists of treating of FMD-infected tissue-culture cells with rabbit FMD antiserum, following which commercial fluorescein-conjugated sheep anti-rabbit globulin is added. The addition of commercial rhodamine bovine albumin to the FMD antisera and to the conjugate enhances the readability of positive reactions against normal rabbit serum controls by eliminating yellowish non-specific fluorescence. The direct method involves fluorescein-conjugated globulin from guinea pigs and bovine FMD antiserum (treated with liver powder and bovine albumin, to which rhodamine has been added to reduce non-specific fluorescence), with FMD-infected cell cultures. With these two techniques, the results indicate that high-passage tissue-culture virus may develop a broad antigenic spectrum. The results to date suggest these procedures may be useful in selection of strains of virus for vaccine production.

In 1962, the mass of antigen-antibody combination was found to be the most essential factor in obtaining fluorescent antibody reactions with foot-and-mouth disease virus (FMDV) that could be evaluated microscopically. A system including probable viral and cellular antigens combining with appropriate antibodies in convalescent bovine serum was developed for practical detection of cattle recovered from foot-and-mouth disease (FMD). The test developed is specific for FMD, but does not discriminate between virus types.

An indirect fluorescent antibody technique using rabbit-rinderpest serum proved satisfactory for demonstration of rinderpest antigen(s) in infected bovine kidney tissue cultures.

C. Diagnostic investigations.

In 1961 preparedness for laboratory assistance in diagnosis of foot-and-mouth disease, and differentiation from vesicular stomatitis and vesicular exanthema, two clinically similar but distinct diseases, were considered of basic importance in the Plum Island program. Work is constantly under way in fundamental explorations aimed toward development of practical, accurate, and rapid means of identification of virus and specific antibodies.

In 1962 there was no report for work on this project.

D. Foot-and-mouth disease vaccines.

In 1961 the optimum conditions with regard to media, quantity of inoculum and time of harvest of virus grown in tissue cultures for large scale production of vaccine have been determined. Virus grown in bovine kidney tissue culture in bottles has produced a satisfactory vaccine for cattle at comparatively low cost and with potentially superior qualities. To date, virus grown in suspensions of trypsinized kidney cells has not been satisfactory for use in vaccine, apparently due to the low virus titers obtained by this method of propagation.

Continuation of studies on the safest methods of inactivation by formaldehyde has resulted in more rigid test procedures than previously were considered necessary. The testing of vaccine in 10 liter lots revealed new conditions which were not obvious when working with smaller experimental lots. In conjunction with these studies a method of testing for residual virus using tissue-culture methods is being explored, with indications that such a method may afford greater assurance of safety than testing in cattle only.

Correlation of measures of potency of vaccine in guinea pigs and cattle is continuing with indications that the response in vaccinated guinea pigs will permit estimation of the probable response in cattle to the same vaccine. A study of this type requires several simultaneous comparative trials in order to determine degree of correlation.

A mineral oil complex is being compared with standard aluminum hydroxide gel as an adjuvant in the vaccine. To date, data indicate the new adjuvant is as good as and possibly superior to the aluminum gel. When the mineral oil complex was used, the antibody response was 100-fold higher in some animals.

Vaccines produced for use in cattle are being tested concurrently in swine. There appears to be a fundamental difference between the two species in vaccine response; swine generally have responded poorly to vaccine produced from virus propagated in tissue cultures. There are some indications, however, that the response in swine may be a function of the adjuvant used in the vaccine and the route of injection of the product.

Comprehensive studies under way in Europe on the duration and extent of immunity in cattle following vaccination with FMD vaccine require long periods of time and many animals. Such investigations are difficult if not infeasible under laboratory conditions. An ADP representative is stationed in Amsterdam, as Chief of the Division's European Mission for Research on Animal Diseases, and such studies are in progress in the Netherlands in cooperation with Dutch researchers. Tests involving 2 herds of Friesian cattle have been in progress for approximately 2 years. Serologic studies in the 2 herds have been continued. Results obtained during the past year have warranted extension of the program to include additional herds. This will afford more significant numbers of animals for challenge with virulent FMD virus and permit correlation of resistance to infection with the index of immunity as measured by virus-neutralization tests of serums.

To obtain sufficient animals for challenge, 10 additional herds have been included in the study. The total number of animals under observation is approximately 400. It is estimated that of this number about 40 annually will become available for challenge. The substantial increase in the number of samples to be tested as a result of this change has justified comparative studies between the virus-neutralization test, depending upon complement fixation, and another test, depending upon changes in pH.

Serums of animals which have experienced two or more annual vaccinations continue to show a high level of antibodies. Two animals showing high levels of antibodies were resistant to challenge with virulent virus, as indicated by absence of generalized infection. Results to date promise eventual development of sound techniques for evaluation of vaccines prepared by various methods, and scientific application of vaccines in the field.

In 1962 cattle vaccinated with inactivated FMD vaccine containing emulsified oil as an adjuvant developed a significantly better protector than cattle receiving a similar product containing aluminum-hydroxide gel as an adjuvant. Study of virus-neutralizing antibodies revealed high antibody levels at 9 months in cattle vaccinated with the emulsified oil preparation, whereas, cattle vaccinated with vaccine containing aluminum-hydroxide gel had low antibody levels at four months. Challenge of these cattle by exposure to live virus showed that the cattle which received the oil emulsion vaccine were better protected.

E. Antigenic variations of foot-and-mouth disease viruses.

In 1961 the susceptibility of mother mice to FMDV began to decline about 3 weeks post-partum until the 5th week when they became almost completely resistant. Substantial reduction of the number of suckling mice 2 days before inoculation of mother mice appears to increase their resistance. Cells from peritoneal exudate of susceptible mother mice exposed in vitro to FMDV have shown no dimunition of phagocytic activity (for yeast) nor was virus absorbed or propagated in these cells. Increasing the number of white cells in the peritonea of mother mice before infection with FMDV did not appear to alter their susceptibility.

In 1962 factors were investigated that might affect the susceptibility of mother mice to FMDV. Virus from tongue epithelium of infected steers and virus from the first few passages in tissue culture produced a low mortality in mother mice, but after 6-8 serial passages in tissue culture the virus killed 40-70% of the mothers. Beginning approximately three weeks post partum, mother mice, which were resistant to the virus before becoming pregnant, gradually became resistant again. The ability of mother mice to respond immunologically is impaired as demonstrated by a hypersensitivity response to bovine serum. The response is lower in both mother mice sensitized seven days post partum and in mother mice sensitized before being bred.

Basic studies under this project have shown that bovine serum antibody may be assayed in the complement-fixation test using guinea pig vesicular fluid as an antigen. Study of sheep serum has shown it to have good complement-fixing antibody and apparently none of the qualities of bovine serum that make CF tests difficult. Comparison of antigens from different sources such as tissue culture and guinea pig vesicular fluid have led to studies to establish antigen standards. Such standards are essential for the serological testing of vaccine antigenicity.

F. Immune response to various types and subtypes of foot-and-mouth disease virus.

In 1961 serological studies were continued on a group of cattle three years after infection and a detectable level of circulating antibody has persisted. Studies have included examination of the animals for latent virus infection to determine whether the persisting antibody level may be due to intracellular virus which has continued to stimulate antibody production. Another approach has been inoculation of pituitary hormones to produce physiological stress which might cause animals to shed latent virus. Although the results to date have been negative, the existence of residual virus in these animals has not been disproved.

In 1962, detailed studies of transfer of immunity from vaccinated cows to their calves have shown this to be through the colostrum only. It has been shown that a calf is born free of serum gamma globulin and only receives it through maternal colostrum. The duration of this passive immunity is dependent on the antibody content of the colostrum received by the calf when it first nurses.

G. Quantity production of foot-and-mouth disease virus.

In 1961 work was initiated on devising methods for rapid and economical production of large quantities of FMDV in surviving kidney-cell suspensions. Steps in the operation being studied included (1) areas of renal tissue providing best viral growth, (2) methods for mincing large quantities of tissue, (3) trypsinizing procedures, (4) types of culture vessels, (5) media, (6) growth conditions, including cell concentration, depth of culture, agitation and alteration, and (7) fragmentation of infected cells for virus release.

In preliminary work, cultures of FMDV, type A, strain 119, have been attained with infectivity titers somewhat higher than the titer considered satisfactory for vaccine production in foreign countries (10^6 infectious units per ml.). Methods of production of large quantities of FMDV, type A, strain 119, in bovine kidney cells cultured on glass have been improved by providing better conditions for the cells for viral growth. This has resulted in a 50 percent increase in susceptibility to infection and in virus yield.

In 1962 methods were developed for rapid and economical production of large quantities of foot-and-mouth disease virus (FMDV) in stationary suspensions of trypsin-dispersed bovine kidney cells in a simple medium. Yields of between 10^7 and 10^8 plaque-forming units (PFU) per milliliter were obtained from serum-free cultures containing approximately a million and a half viable trypsin-dispersed cells per milliliter. Yields of up to $10^{6.9}$ PFU were obtained from simple cultures of finely minced calf kidney tissue provided cell debris was removed from the minced particles by trypsinization.

H. Microcinematography of infected cells.

In 1961 tissue cultures infected with type A, strain 119, FMDV were photographed. The cytoplasm of infected cells contracts around the nucleus leaving small branching streamers of protoplasm attached to the glass resulting in a bush-like or tree-like alteration of the cytoplasm. The term "arborization of the cytoplasm" was applied to this stage of the degenerative change. Normal cells undergoing mitosis also contract but their cytoplasm fails to undergo arborization. After the degenerating cells have undergone arborization, they undergo a form of activity described as "boiling", and then detach from the glass. Critical evaluation of preliminary films indicated the need for substantial modifications of the equipment to provide longer exposure time at higher magnification. This was accomplished, and considerable experimentation with different methods of film processing was made to provide high-quality film.

In 1962 improvements in microcinematographic technique have resulted in production of films satisfactory for demonstration and possible distribution. Microcinematographic studies have been made on growth of normal primary calf-kidney cultures and cultures infected with foot-and-mouth disease (FMDV) and rinderpest virus. Films prepared have been shown at scientific meetings. The phenomenon of cell survival was noticed and confirmed in the course of the above studies. A small portion of the cell population in primary calf-kidney cultures resists the action of FMDV. The cells not destroyed by the virus continue to multiply. Virus in low titer persists in the surviving cultures but a small-plaque moiety becomes predominant. Studies on changes developing in virus populations persisting in surviving cultures are being made.

I. Pure stable lines of culture cells.

In 1962, a manuscript was prepared with abstract as follows: Continuous culture of lamb testis cells on glass and in agitated fluid suspension cultures has been achieved several times. No obvious cell alterations occurred during more than 40 serial passages. The culture system appeared to be well suited for production and assay of foot-and-mouth disease and some other animal viruses. Several additional established cell lines from outside sources were tested for susceptibility to foot-and-mouth disease virus with negative results.

J. Purification of foot-and-mouth disease virus.

In 1961, a rapid method of centrifugation for purifying FMDV was developed which combined isodensity separation immediately below a moving zone separation. A preformed density gradient of cesium chloride enabled the virus to band into a narrow zone within 4 hours at 37,000 rpm in a swinging bucket tube. The viral light-scattering zone when removed at a concentration of 8-fold had 47± 16% of the original infectivity and contained virus particles as revealed by electron microscopy. The cesium chloride isodensity value of FMDV of 1.43 ± 0.01 g/ml was significantly higher than that of protein contaminants.

Combinations of basic types of centrifugation include sedimentation of FMDV through an interface formed by an aqueous phase and an immiscible organic fluid. Separation of virus from contaminants appears to depend upon hydrated particle densities and specific denaturation by organic fluids, in contrast to the dependence upon anhydrous particle densities in cesium chloride gradients.

Certain viruses, particularly bacteriophages, are stable only in the presence of magnesium ions. This ion, however, was found to have no stabilizing effect and possibly even detrimental to FMDV infectivity. This observation prompted use of the chelating agent, sodium ethylene diamine tretraacetate (EDTA) to remove bivalent cations from the highly purified virus described above. In 1% EDTA infectivity was essentially constant over a 55-day period at -60C; longer periods have not been investigated. EDTA can be readily removed by dialysis.

In 1962, a new ultracentrifugation technique termed organic interface centrifugation was perfected. It combines in one run, moving boundary centrifugation, isodensity purification and organic extraction. Foot-and-mouth disease virus is being purified and concentrated from infectious tissue culture fluids by procedures incorporating alcohol precipitation, organic extractions, cesium chloride density gradient centrifugations and organic-interface centrifugations. The average weight of virus obtained from 10 liters of fluid was 100 micrograms based on electron microscope counts. This work will be continued until foot-and-mouth diseease virus of at least 95% purity is obtained. Progress has been limited by the lack of a virus production unit at Plum Island.

K. Chemical and physical characterization of foot-and-mouth disease virus.

In 1961, foot-and-mouth disease virus (FMDV), type A, from infected guinea-pig foot-pad vesicular fluid was examined for fine structure in both phosphotungstic acid and uranium-shadowed preparations on carbon coated grids. Phosphotungstic acid penetration was less than that reported with many other viruses,

hence shadowed specimens were also used extensively to study the structure. Regular polyhedral models of 12, 20, 32, and 42 subunits were constructed and analyzed for distinguishing features exhibited by both virus and model. Mixtures of FMDV and bacteriophage which have approximately the same diameters, revealed that FMDV had smaller and more numerous subunits. The icosahedral model with 42 subunits was favored over the modified dodecahedral model of 32 subunits for FMDV, although there was no unequivocal evidence that these represented the ultimate structure of FMDV. A carbon shadowing device was modified to facilitate studies of ultra-structure. The improvement consisted of a tungsten metal spring for advancing the pointed carbon rod toward the cavity in the blunt rod.

In 1962, foot-and-mouth disease virus was compared in the electron microscope with a bacteriophage and Turnip yellow mosaic virus (32 subunits). The number of subunits in foot-and-mouth disease virus exceeded that of the bacteriophage, but the exact number could not be determined. Complications in ultracentrifugation calculations introduced by the use of variously shaped cells were simplified by using higher mathematical functions in tabular form. Tabulation of log values required for sedimentation coefficients permits rapid calculation of the minimum s-rate for sedimentation for a known volume.

L. Interaction between foot-and-mouth disease virus and host cells.

In 1961, cultured bovine cells, partially depleted of endogenous metabolites and maintained on a medium with glucose as the sole organic material, were examined for acid production by known chromatographic procedures. The kinds of acids detected and their rates of production were the same for both normal and infected cells. Lactic acid constituted about 90% of the total acidity with acetic acid being the only other acid identified during the 12-hour post-starvation period studied. Concentration of acetic acid was highest during the initial recovery period of the starved cells. Cells infected with FMDV yielded comparable results although acetate production appeared to persist for longer periods of time. No acids of the tri-carboxylic acid cycle were detected in either infected or uninfected cells.

Cells partially depleted of endogenous nutrients utilized pyruvate at a rate 2.5 to 4 times greater than glucose. The pathways of pyruvate metabolism were not clearly defined. Pyruvate was not appreciably metabolized oxidatively since oxygen uptake could account for only 6-29% of the pyruvate consumed and malonate only slightly decreased its utilization. Substrate pyruvate did not form lactate and was used to a greater extent as glucose concentration decreased. Glucose utilization was independent of pyruvate concentration. Virus infection did not change the rate of pyruvate utilization in contrast to the increase found in glucose uptake. Lactalbumin hydrolyzate did not yield lactic acid of itself but did stimulate glucose uptake and corresponding lactate formation. Acetate was not oxidized nor was lactate produced. Cells uninfected with FMDV maintained the same pattern of glucose metabolism as did uninfected cells.

Thin sectioning for electron microscopy has been started with the examination of sedimented cells from normal and FMDV-infected bovine kidney cultures as well as with tissues infected with rinderpest virus. Training of a technician in routines of fixation, embedding, sectioning and examination of the thin sections has been started. Studies are planned and in progress for following the synthesis of FMDV and of rinderpest virus in cultures.

In 1962 further chemical investigations of organic acids synthesized by bovine kidney culture cells grown in media with and without serum showed lactic acid to be the major component, accounting for 86 to 90% of the total, while acetic and pyruvic acids accounted for 7 to 9% and 3 to 6% respectively. Foot-and-mouth disease virus-infected cultures, independent of medium, produced more of these acids than uninfected cultures. Studies with cultures partially depleted of endogenous nutrients and exposed to serum-free medium indicated that acetic and pyruvic acids may be derived from an endogenous substrate other than glucose. The contribution of lactic acid to total acidity increased with increasing glucose concentrations.

The above chemical and other metabolic studies of glucose metabolism in bovine kidney culture cells were confirmed by radioisotope tracer studies. Extracellular acids account for nearly all of the C^{14} with over 90% residing in lactic acid. Intracellular isotopic activity, 1 to 3% of the total, was distributed among the cold trichloracetic acid soluble pool, hot trichloracetic acid, lipid and protein fractions.

Purine and pyrimidine analogues were studied for possible inhibition of foot-and-mouth disease virus synthesis in bovine kidney culture cells. Eight analogues at 250 ug/ml did not alter cellular metabolism measured in terms of oxygen consumption and glucose utilization. When tested for inhibition of virus synthesis 8-azaguanine, dithouracil and 5 bromouracil sometimes decreased the amount of recoverable virus. The latter compound gave the most consistent decreases, and the inhibition was not prevented by adding natural pyrimidines, uracil and thymine. Interpretations are not clear-cut because controls on virus stability in vitro with the analogues showed that 8-azaguanine and dithouracil had no effect, while 5 bromouracil caused significant losses in activity.

M. Genetic biochemistry of foot-and-mouth disease virus.

In 1961 the presumption that FMDV splits off infectious ribonucleic acid (RNA) when heated has been substantiated. The tedious job of removing all ribonuclease (RNase) from virus preparations was accomplished. The resulting RNase-free virus yielded infectious RNA when heated at 61 and 85 C. Bentonite has been found to eliminate the last traces of RNase from phenol-derived RNA. Such RNA may be stored for at least 3 months at -60C with undiminished infectivity. Heretofore, phenol-derived RNA retained full infectivity only when stored in liquid nitrogen.

The precision of the plaque assay for FMDV-RNA was determined. Randomizing older data for 100 replicate platings of RNA in two ways, i.e., into groups of 5 and 10, gave respective ranges with one standard deviation of 27.2 ± 21% and 54.3 ± 16%. New data for 50 replicate platings gave a range of 17.5 ± 27%. Thus, plaque counts ranging between 17 to 54 are known to a precision of 27% or better 65% of the time. The plating efficiency of FMDV-RNA is increased nearly 10-fold by inclusion of slightly soluble substances in the inoculum. The best substance found thus far is Attasorb.

Work is continuing on an apparent RNase inhibitor in calf-kidney cell cultures. The RNase-like activity of cell extracts inactivating FMDV-RNA is potentiated markedly by heating, pH changes and by the protein-protein dissociating agent, p-chloromercuribenzoate (pCMB). The optimal temperature range for thermal potentiation is 55 to 65 C with activity increasing as much as 1000-fold. If sodium dodecylsulfate (SDS) is present prior to heating, the RNase-like activity of cell extracts is potentiated to a lesser degree. This is in accord with the finding that virus heated in the presence of SDS yields infectious RNA, with the hot SDS inactivating environmental RNase. Transient acidification or alkalization of cell extracts increases their RNase-like activity by 10-fold or greater. pCMB also effectively increased the ability of cell extracts to destroy RNA infectivity. Dissociated inhibitor and RNase do not recombine readily.

In 1962 kinetic curves for the thermal inactivation of FMDV-RNA, freed with bentonite of the last traces of ribonuclease, were determined at 7 temperatures over a 3 hour period. Such RNA is not pure and contains much cellular RNA and DNA. The rates were first order with 1.5-hour survivals of 0.095, 0.85, 0.45, 0.25, 0.08 and 0.006 at 1°, 26°, 37°, 45°, 55° and 61°C, respectively. Survival was only 0.001 after 10 minutes heating at 85°C. The primary structure, secondary structure and reactivity of infectious FMDV-RNA obtained from pure FMDV is under investigation.

N. Effects of chemical and physical environments of foot-and-mouth disease virus.

In 1961 a rate study of the inactivation of the virus with AEI (acetylethylenimine), BPL (betapropiolactone), and ETO (ethylene oxide), was performed at 23 C. To 250 ml. volumes of A-119 virus in the 89th tissue-culture passage 0.05% AEI, 0.05% BPL, or 0.5% ETO was added. Samples were taken at 2-hour intervals in the critical period of inactivation. In innocuity tests in pairs of cattle when 2 ml. of each sample was given IDL, the results were as follows: AEI in a concentration of 0.05% inactivated the virus in 22 to 24 hours, BPL in a concentration of 0.05% inactivated the virus in 12 hours, and ETO in a concentration of 0.05% inactivated the virus in 16 hours. The cattle which showed no signs of FMD were challenged after 14 days with $10^{6.2}$ bovine ID_{50} by the intramuscular route. In the cattle that had been injected with virus inactivated by AEI in a 24-hour exposure, no primary or secondary lesions of FMDV were found in the 14-day observation period. In the cattle inoculated with virus in the presence of BPL for 12 hours, both tongue and foot lesions developed during the post-challenge observation period. Injection

of virus in the presence of ETO for 16 hours permitted development of tongue and foot lesions in the challenged cattle. It appears from these studies that AEI is superior to BPL and ETO as an inactivant. Ancillary tests such as virus-neutralizing capacity of the serum of chickens injected with 1 ml. of the same preparations as given to the steers, and the virus-neutralizing capacity of adult mouse serums after injections with the inactive viral preparations confirm the foregoing assumption. The following titers in PFU/ml have been obtained with A, O, C, SAT-1, SAT-2, SAT-3, and Asia-1, respectively, after 89, 22, 22, 8, 8, 8, and 10 tissue-culture passages: 8.0, 7.8, 7.6, 8.2, 7.7, 6.3, and 7.3, in that order. The titers in mice as $LD_{50/ml}$ approximate the tissue culture PFU/ml.

In 1962, studies on chemical inactivation of foot-and-mouth disease virus (FMDV), 0.05% acetylethyleneimine (AEI), at a temperature of 23°C for 24 hours, inactivated FMDV propagated in tissue cultures. The effects of beta-propiolactone (BPL) were also studied and were found to be less reliable than AEI. FMDV preparations inactivated with AEI retain the greater part of their antigenicity while BPL is more severe on viral antigenicity as tested in cattle, chickens, and mice. Cattle used in the infectivity studies were injected in the tongue and were later shown to be immune when challenged by the intramuscular route with concentrations of virus as low as 10,000 bovine ID_{50}.

Twenty nine cationic and anionic surface active agents were tested for virucidal effect against Type O-M11 FMDV. Only one chemical, methyl ethyl isoquinilinium chloride, was capable of destroying 3-4 logs of viral infectivity in 2 hours at 28 C, whereas others only inactivated between 1-2 logs of virus under the same conditions.

O. Preservation of foot-and-mouth disease virus.

In 1961 tissue culture-propagated virus and virus in bovine tongue-tissue suspensions were used for storage in three replicate experiments. To lots of tissue cultured A-119 virus in the 88th passage 5% gelatin, 5% sucrose, or 0.2% cysteine were added, respectively. Antibiotics in concentrations of 1000 units of penicillin and 1 mg. of streptomycin were added per ml. Samples of each preparation were stored at 37, 23, 4 and -50 C in flame-sealed ampoules. Lots of 10% suspensions of tongue tissue from cattle infected with A-119 virus in the 9th and 10th bovine passages were supported with 2% gelatin, 5% sucrose, 50% normal bovine serum, L.C. fluid (tissue culture medium), or tryptose phosphate broth. Antibiotics as described above, were also added. Samples of the preparations in flame-sealed ampoules were stored at 37, 23, 4 and -50 C. Infectivity studies of the various preparations were conducted in suckling mice. At the end of a year, it was evident that virus of tissue-culture origin with or without additives, was more stable at 37, 23 and 4 C than was virus in tissue suspension. Both forms of the virus retained the approximate original titers when held at -50 C for a year. Tissue-culture virus survived at least 7 days at 37 C, for 8 weeks at 23 C, and 12 months at 4 C. Virus in tissue suspension survived for 2 days at 37 C, 2 weeks at 23 C and 6 months at 4 C. The additives gave little if any supporting effect except in tissue suspension supported by 50% bovine serum at 4 C.

Cysteine had an apparently harmful effect. The freeze-drying was performed on an Edward's freeze-drying machine. It was established previously that a condenser temperature of -50 C must be maintained in the primary phase of drying with a vacuum of 150 mm or less of mercury throughout a period of 20 hours, followed by a vacuum of 80 mm for the secondary phase of drying over P_2O_5 for 4 hour period at 23 C. Virus from tissue cultures and in tissue suspensions was dried and stored in 10 ml ampoules in 4 ml. amounts. Additives in the following concentrations were used singly or in combination with tissue culture virus: 5% sucrose, 2% gelatin, 2% dextrin, 2% sucrose, 5% skim milk powder, 1% glutamate, and 5% normal bovine serum. Tryptose phosphate, L. C. fluid, 5% normal bovine serum, 5% glutamate, and 5% skim milk powder were used singly or in combination with virus in tissue suspension. All vials were flame-sealed under vacuum after drying. Titration of samples for infectivity was conducted in suckling mice. In the presence of perfect mechanical conditions it has been possible to dry the virus with only 0.5 to 1 log loss in infectivity. Tissue-culture virus without supportive preparations dropped in titer from 0.5 to 1 log of virus following storage for one year. However, for best storage 5% skim milk powder or a combination of 5% sucrose, 5% bovine serum, and 1% glutamate concentration should be added. Virus in tissue suspensions stored well for a period of a year with tryptose phosphate or L.C. fluid as diluents. Data is not complete for all lots of freeze-dried virus in storage for a year.

In 1962, type A-119 foot-and-mouth disease virus (FMDV) was propagated in tissue cultures and in the dermis of cattle tongues. Various proteins, carbohydrates, or salts of amino acids were added to both types of viral preparations. Samples of these materials in 4-ml. amounts in flame-sealed ampoules were held at 37, 4, 23, and -50 C for periods up to a year. Virus produced in tissue cultures survived at least 7 days at 37 C, for 8 weeks at 23 C, and 12 months at 4 C. Virus in tissue suspensions survived 2 days at 37 C, for 2 weeks at 23 C, and for 8 months at 4 C. None of the additives significantly increased the storage survival. Virus from both sources, with or without additives, was stable for a year at -50 C. All infectivity tests were conducted in mice.

Similar preparations of Type A-119 FMDV were prepared with various additives such as proteins, carbohydrates, or salts of amino acids. These preparations were freeze-dried in 4-ml. amounts in ampoules in the Edward's freeze-dryer and stored for a year at 4 C. In most instances the over-all loss of virus in processing and storage did not exceed a log in infectivity. There was no apparent difference in storage stability between the two sources of virus and the additives apparently did not appear to influence the storage qualities.

P. Rinderpest.

In 1961 some dogs inoculated with rinderpest virus developed antibodies to rinderpest and resisted challenge with distemper virus. Cattle inoculated with measles and distemper viruses failed to develop antibodies against rinderpest virus. Inoculation of monkeys with distemper and rinderpest viruses and subsequent challenge with measles virus will complete the study.

Three strains of rinderpest virus passaged in tissue cultures have been reduced in virulence for cattle. All strains tested have been shown to share common antigenic factors. Some such strains may be useful as immunizing agents.

Although some progress has been made in electron microscopy studies, the virus particle of rinderpest has not yet been identified. It has been found that the virus is unstable in butanol and other lipid solvents. The virus was readily precipitated by centrifugation, but after being pelleted it could not be fully resuspended. Thus, purification procedures employing lipid solvents and pelleting have not been applicable. This ruled out, for the present at least, the possibility of using lymph nodes as a source of virus because of their relatively high content of impurities. Progress has been made, however, in concentrating and purifying tissue-culture virus. When the virus was found to withstand high salt concentration, it was possible to zone it by centrifugation in cesium chloride density gradients with nearly complete recovery of infectivity, 100-fold concentration, and the elimination of much debris. Such material after dialysis to remove salt, revealed a few virus-like particles, approximately 100 millimicrons in diameter, but they were too few to permit correlation with infectivity. The virus was concentrated about 100-fold by dialysis against polyethylene glycol, and its infectivity was unaffected by polyethylene glycol.

In 1962 relationships of the viruses of human measles, canine distemper and rinderpest were studied. Cattle injected with measles or distemper virus were not protected against challenge with rinderpest virus, although distemper antibodies were found in cattle injected with that virus. Each of the viruses elicited antibody response in an alien host. Puppies injected with either measles or rinderpest virus were protected against distemper.

Work was continued on the attenuation of the Kabete strain of rinderpest virus in tissue cultures. The modified virus retained sufficient antigenicity to immunize cattle, and in limited studies, it produced only limited clinical response. This strain of virus was inactivated at pH 2 and 12; but at pH 3, the virus remained viable for 5 minutes, and at pH 11, for 20 minutes. Virus-cell relationships were studied by electron microscopy. From this work, it is apparent that rinderpest virus develops in the mitochondria of infected bovine kidney cells in cultures.

Cross-immunity studies were made with one strain of bovine virus diarrhea and rinderpest. Calves recovering from experimental reinfection with virus diarrhea were susceptible to challenge with rinderpest virus, and serum from animals with virus diarrhea did not cross-react with rinderpest virus. Calves experimentally immunized against rinderpest, did not develop antibodies to virus diarrhea virus, and such animals were susceptible to challenge with virus diarrhea virus. These results indicate lack of serological or immunological relationship between the two viruses.

Q. Transmission of foot-and-mouth disease virus in semen.

In 1961 guinea pigs, bulls and heifers were used in preliminary studies. Guinea pigs and steers were used for infectivity studies. Type A, strain GB, FMDV was used in the studies in guinea pigs and types A, strain 119, and O-M11 were used in cattle.

Virus was found in the urine, testicles, vas deferens and epididymus of male guinea pigs 48 hours after inoculation via the metatarsal pads, but was not found in the seminal vesicles. The titer of virus in the vas deferens was higher than that observed in the other tissues and fluids. Virus was detected in the testicles 24, 48 and 72 hours after inoculation. Female guinea pigs showed lesions of FMD 120 hours after instillation of virus in the vagina or rubbing on the vulva. Other female guinea pigs failed to develop FMD when virus, diluted 1:10 with milk or egg yolk semen extenders, was instilled into the vagina. Control animals inoculated with diluted virus intradermally in the foot pads developed lesions within 24 hours. In limited trials, urine from infected male guinea pigs produced lesions when inoculated into the foot pads, but failed to produce FMD infection when instilled in the vagina of susceptible guinea pigs.

Two bulls were inoculated on the tongue with Type A, strain 119, FMDV and slaughtered 24 hours later, when signs and lesions of infection were evident in only one of the two animals; however, both developed viremia (titers of $10^{4.8}$ and $10^{4.5}$ ID_{50}/ml, respectively). The testicles, epididymus and lining of the bladder from both animals contained infective virus. Virus was not found in the seminal vesicles or lining of the urethra from either bull, however, infective virus was demonstrated in the vas deferens from the bull which developed clinical signs of FMD prior to slaughter.

Heifers in oestrus and not in oestrus were used. The virus was prepared from infected bovine tongue tissue in various suspending fluids. Approximately 3.0 ml. of virus suspension was instilled in the vagina at a depth of about 12 inches, using precautions to prevent contamination of the vulva and surrounding area. Of two heifers receiving a bovine semen-virus preparation, one, which had been in oestrus 4 days prior to vaginal exposure, developed FMD within 4 days. The heifer not in oestrus did not develop clinical evidence of FMD and was susceptible to subsequent inoculation. Four heifers received the virus diluted with egg-yolk extender. One of three heifers in oestrus at time of exposure developed clinical FMD. The other two heifers remained free of evidence of infection and were susceptible to a subsequent inoculation. One heifer not in oestrus at the time of vaginal exposure developed signs and lesions of FMD 5 days later. Two additional heifers not in oestrus developed FMD within 4 days after vaginal exposure with 10 per cent suspension of Type O, strain M-11, virus diluted equally with bovine semen.

In 1962 semen samples were collected at various intervals after inoculation from 8 FMD-infected bulls by use of an electroejaculator. Urine and blood samples were also taken. Infectivity studies were conducted in mice and steers. FMDV (A-119 or O-M11) was found in the semen and urine of bulls as early as

12 hours and in blood as early as 6 hours after inoculation. During convalescence, virus was found in semen and urine for as long as 7 days and in the blood for as long as 4-3/4 days after inoculation. The titer of the virus in the semen usually was higher than in urine and sometimes higher than in the blood. The pH of the urine usually shifted from alkaline to acid about 2 days after inoculation and then returned to alkaline in about a week as the bulls recovered from acute infection. The pH of semen varied, but remained nearly neutral throughout the course of the disease.

R. Survival of foot-and-mouth disease in meat and meat products.

In 1961 studies previously reported demonstrated that FMDV may be found in lymph nodes, hemal nodes, bone marrow and large blood clots in carcasses of infected animals ripened at 4 C for 72 hours. The studies were extended to determine if virus also could be detected in bone marrow and lymph nodes of animals slaughtered at times after the initial stages of infection.

Virus was demonstrated in fresh rib bone marrow of steers for as long as 3 days after inoculation with FMDV A-119. Virus was not found in bone marrow of steers slaughtered 4, 5 and 9 days after inoculation with FMDV A-119, nor was virus found in bone marrow of a steer 7 days after inoculation with FMDV type SAT-3.

All 7 types of FMDV were used to determine the length of time after inoculation that the various viruses could be detected in lymph nodes. The number of days after inoculation that the various types and strains of infectious FMDV survived in lymph nodes of infected steers was as follows: 9 days for O-M11, C-149 and Asia 1; 11 days for A-119 and SAT-3, and 13 days for SAT-1 and SAT-2. However, on the 13th day post-inoculation, even though the test steers did not become clinically ill from the material inoculated from A-119 and C-149 infected donors, some of the test steers were sufficiently immunized by the material to resist subsequent challenge and had significant virus-neutralization indices, 3.7 and 5.5, respectively.

The titer of FMDV in the head and body lymph nodes of infected donor steers was compared on various days after inoculation, using 3 strains of FMDV (SAT-1, O-M11 and A-119). As might be expected, since the steers were infected by tongue inoculation, the titer of the virus was usually slightly higher in the head lymph nodes (mandibular primarily) than in the body nodes. For the first 3 days after inoculation the virus titers in the lymph nodes varied from 1.3 to 4.5, with an average of 3.2 (bovine ID_{50} or mouse LD_{50} per ml. based on 15 titrations). At the 7th, 8th and 9th days after inoculation, the titers varied from 1.3 to 3.0, with an average of 2.4 for 5 observations. No virus was demonstrated in 11 other samples tested. Virus titers as high as 5.6 have been obtained from the blood of steers on the third day after inoculation with FMDV A-119, and 7.8 or higher from infected tongue tissue, while the titer in lymph nodes has not exceeded 4.5

In 1962 ground meat, composed of lymph nodes and muscle tissue, in a ratio of 1:10, was prepared from FMD-infected steer carcasses stored at 4 C for 72 hours. The ground meat was stored at 4 C, unsalted and salted (4% NaCl). When fluids were expressed from unsalted meat, FMDV was found as long as 11 days, but could not be detected in salted meat stored for more than 4 hours. However, when the lymph-node fragments were sorted from the ground meat and tested, virus was found in salted meat for as long as 17 days.

The titer of FMDV in lymph nodes declines during storage; 34 samples gave an average titer of $10^{3.8}$ (fresh) and about $10^{1.6}$ after 9-10 days storage. FMDV in lymph nodes taken during the pre-clinical stage of the disease apparently does not survive as long during storage as virus in lymph nodes taken after the disease is well established.

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PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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AREA NO. 9 - FOOT-AND-MOUTH DISEASE AND OTHER EXOTIC DISEASES OF SWINE

Problem. Foreign diseases such as foot-and-mouth disease, African swine fever, and Teschen disease, that occur elsewhere in the world, constitute calculable potential threats to the swine industry of the United States. Foot-and-mouth disease is of particular importance because the disease frequently occurs primarily in swine from which it spreads to other susceptible species, such as cattle and other ruminants. African swine fever, which until recently was confined to wild and domestic pigs in Africa, has spread to Portugal and Spain. The disease is of special concern because of its resemblance to hog cholera, with which it may be confused. Moreover, mortality from the disease approaches 100 per cent, and there is no specific preventive vaccine. Teschen disease, which causes widespread inapparent infections and occasional involvement of the central nervous system, is another of the foreign diseases to be guarded against. A disease indistinguishable from Teschen disease has appeared in England in recent years. Despite all precautions, any of these diseases may occur in the United States, as likely as not through the medium of modern, rapid international transportation. The Plum Island Animal Disease Laboratory is engaged in studies of foreign diseases of swine, for the purpose of developing information for increased protection of the Nation's swine industry.

USDA PROGRAM

The Department has a continuing long-term program involving veterinarians, biochemists, microbiologists, and pathologists, engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 5.6 professional man years. This effort is divided among sub-headings as follows:

Foot-and-Mouth Disease of Swine 1.0 at the Plum Island Animal Disease Laboratory, Plum Island, New York.

African Swine Fever 4.6 at the Plum Island Animal Disease Laboratory in cooperation with the East African Veterinary Research Organization, Muguga, Kenya, and in connection with a P.L. 480 project in Madrid, Spain, where the equivalent of $97,550 has been made available to the Spanish Ministry of Agriculture over a 3-year period.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

Owing to prohibition of experimentation with these diseases in the United States, except at the Plum Island Laboratory, no work in this area is being done in this country at State Experiment Stations or by American industry.

REPORT OF PROGRESS FOR USDA

A. Foot-and-mouth Disease

During 1961 at the Plum Island Animal Disease Laboratory, immunological investigations of foot-and-mouth disease of swine were carried out. The response of a group of swine to infection with foot-and-mouth disease virus was determined. These studies confirmed the concept that fundamental differences exist in immune response between swine and cattle. Swine respond to infection with rapid appearance of antibody which drops to low levels within four months, permitting some of the animals to become reinfected by contact with infected animals. Vaccination of swine with formalized tissue culture vaccine has generally been unsatisfactory.

During 1962 research effort has been directed toward the development of assay procedures suitable for study of antibody in swine serum. Experimentation has been directed toward determination of the antibody response after infection. Pretreatment of swine serum with dilute solutions of formaldehyde completely removed the procomplement activity and permitted detection of FMD antibody by complement-fixing methods. This agreed with previous findings in work with African swine fever virus. In some trials, however, it has been observed that complete fixation did not occur unless normal bovine serum was included in the mixture.

B. African Swine Fever

In 1961 investigations were continued in Kenya in cooperation with the staff of the East African Veterinary Research Organization. Bush pigs, as well as wart hogs, have been found to be inapparent carriers of the disease. Three virus isolations were made from 76 bush pig specimens examined.

Since African swine fever has become widespread in Spain and Portugal there is even greater need for methods of differentiating ASF from hog cholera, and for a better understanding of the mechanism of infection with ASF virus and other aspects of the disease that are important in prevention, control, and eradication.

An improved and simplified method of preparing pig leukocyte cultures has made possible production of uniform cultures for routine use in assaying ASFV. These cultures are being used to measure rates of virus inactivation occurring at various levels of pH and temperature.

By alternate passages in pig leukocyte and chicken embryo cell cultures it has been possible to adapt several strains of ASF virus first to chicken embryo cells and then to a line of pig kidney cells.

The enhancement of the complement-fixation reaction by the addition of normal bovine serum has made possible the detection of complement-fixation antigen in extracts of spleen and lymph nodes from pigs infected with ASFV. Additional work is required to establish the reliability of the technique.

In preliminary studies, certain of the antigenic components, active in the agar-gel precipitin test, are destroyed by proteolytic enzymes while others are not. The antigens also vary in their heat stability.

In 1962 epizootiological studies have continued to determine the incidence of carriers of the virus among wild species of animals in Kenya. In addition, work is being conducted to determine the number of types of virus in order that a logical approach may be made to develop immunizing agents.

Work has continued to adapt various stains of ASF virus to cell cultures. Methods for adapting ASFV to cell cultures have been established and several isolates have been serially propagated in chick embryo cells and a line of pig kidney cells. There has been evidence of some modification of the virus following passage in cell cultures. More isolates will be adapted and serially propagated in cell cultures to obtain one or more attenuated strains which may serve as immunizing agents against all strains of ASFV.

Work is also continuing to develop means of propagation of quantities of ASFV by cell culture methods. Quantities of the virus will be especially useful in studies on virus inactivation and production of viral antigens. Serological investigations have continued and it has been shown that swine which survive the acute stage of ASF develop complement-fixing antibodies against antigens in infected tissue culture. The development of a means of serological diagnosis is a significant advancement and one which should supplement diagnosis by the hemadsorption test which was developed several years ago. Diagnosis, however, by serological methods would permit a more rapid means of distinguishing ASF from hog cholera than may be done with the present hemadsorption technique.

The epizootiological studies have shown that bush pigs, wart hogs, and porcupines may be inapparent carriers of ASFV.

The work under the P.L. 480 project in Spain is primarily on diagnostic studies. The hemadsorption test was applied to specimens from 243 suspected outbreaks of ASF. Of these, 172 were positive, 67 were negative, and 4 were toxic to the leukocyte cultures. In these investigations the hemadsorption effect was specific for African swine fever and bacterial contamination did not interfere with the test.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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AREA NO. 10 - PARASITES AND PARASITIC DISEASES OF CATTLE

Problem. The cost of parasitic diseases to the cattle industry of the United States is estimated to be in excess of $400 million annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy cattle, insure adequate supplies of parasite-free beef for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a more prosperous agriculture and the national economy.

USDA PROGRAM

The Department has a continuous long-term program involving biochemists, microbiologists, parasitologists, pathologists and veterinarians engaged in both basic and applied studies directed to the development of measures for the solution to the high and extremely costly incidence of parasitism in cattle. Research is being conducted on parasitic diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 17.7 professional man-years. This effort is divided among subheadings as follows:

Ecological Factors Influencing Nematode Development 1.1 at the Regional Animal Parasite Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Effects of Mixed Helminth Infections 2.0 at the Regional Animal Parasite Laboratory, Auburn, Alabama.

Acquisition of Parasites from Pastures 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Effect of Pasture Mixtures and Pasture Management on Control of Internal Parasites 1.5 at the Regional Animal Parasite Laboratory, Auburn, Alabama, and through informal cooperation with the Georgia Experiment Station, Experiment, Georgia.

Winter Coccidiosis (Bloody Scours) 1.0 at the Regional Animal Disease and Parasite Laboratory, Logan, Utah, and under a cooperative agreement with the Montana Agricultural Experiment Station, Bozeman.

Influence of Diet and Nutrition of Cattle on Roundworm Parasitism 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Artificial Propagation of Protozoan Parasites 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Host-Parasite Relationships of Coccidia 1.0 at the Regional Animal Parasite Laboratory, Auburn, Alabama.

Ecology and Immunology of Lungworms 1.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Clinical and Physiological Aspects of Roundworm Parasitism in Cattle 0.1 at the University of California, Davis under a cooperative agreement with the USDA.

Investigations of Trichomonad Parasites 1.0 at the Regional Animal Disease and Parasite Laboratory, Logan, Utah, and under a cooperative agreement with the Utah Agricultural Experiment Station, Logan.

Host-Parasite Relationship of Intestinal Worms Cooperia spp. 2.0 at the Regional Animal Parasite Laboratory, Auburn, Alabama.

Anaplasmosis 4.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and through memoranda of understanding and other arrangements in cooperation with State Experiment Stations in California, Illinois, Louisiana and Nevada, the State Veterinarian of Tennessee, the USDA Entomology Research Station, Kerrville, Texas, the Delta Branch Experiment Station, Stoneville, Mississippi, and a large cattle ranch in Virginia and in Wyoming.

Investigations on Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle, are under way through a PL 480 Grant, at the School of Veterinary Faculty, Montevideo, Uruguay.

Investigations on the Pathogenesis of Lesions Produced by the Leech, Limmatus nilotica are under way at the Hadassah-Hebrew University Medical School, Israel, under a PL 480 grant.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 21.7 professional man-years divided among subheadings as follows: Ecological factors influencing nematode development 2.4; mixed helminth infections 4.4; acquisition of parasites from pastures 3.9; winter coccidiosis (bloody scours) 1.0; influence of diet and nutrition of cattle on roundworm parasitism 0.9; clinical and physiological aspects of roundworm parasitism in cattle 1.1; anaplasmosis 6.9. Nine southern and four western States and

the USDA cooperate in regional research (S-21, Gastrointestinal Parasites of Ruminants, and W-35, Nematode Parasites of Ruminants) to determine the Ecological Factors Influencing Nematode Development. The two regional projects on internal parasites of ruminants (S-21 and W-35) provide a basis for cooperation on the project Mixed Helminth Infections. The factors which influence the Acquisition of Parasites from Pastures by cattle are being studied in the South (Regional project S-21). Georgia, Louisiana, and Puerto Rico are studying conditions such as moisture on grass blades, sunlight striking the blade, height and type of grasses. Studies conducted in Mississippi include the effect of different seasons, the type of seasonal grasses and soil sterilants on parasite larvae. Arkansas,Georgia, Louisiana, and Texas are cooperating in regional research (S-21) to evaluate factors of parasite control. Examples are management, rotation, stocking of pastures, supplemental feeding, water, soil types, drainage and shade. Georgia, Mississippi and Oregon are determining which are the best grasses or mixture of grasses for parasite control. Studies in cooperation with the USDA are in progress in the North Central Region and the Western Region on contributing conditions for Winter Coccidiosis (bloody scours). The evaluation of specific food elements, carbohydrates, fats, minerals, proteins and vitamins, are being studied in the North Central Region and Southern Region (S-21) in cooperation with the USDA to determine the Influence of Diet and Nutrition on Roundworm Parasitism. The Southern Region (S-21) and Western Region (W-35) are cooperating with the USDA to determine the Clinical and Physiological Aspects of Roundworm Parasitism in Cattle. Research studies on Anaplasmosis are in progress in the north central, southern, and western regions in cooperation with the USDA.

Industry and Other Organizations especially chemical companies, are engaged in research on the formulation of compounds and the exploration of chemicals that may be used safely as parasiticides. Most of the companies engaged in this kind of research utilize their own personnel, facilities and funds. The efforts and results of the work are generally considered as confidential since the ultimate goal of the companies is to produce saleable products. It is estimated that approximately 50 professional man-years are devoted to the work.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Ecological Factors Influencing Nematode Development.

In 1961 at the Animal Disease and Parasite Research Division's (ADP) Regional Animal Parasite Laboratory, Auburn, Alabama, the research showed that eggs are laid by Cooperia oncophora in various stages of development and their hatching times are correspondingly varied. Measurements, stages of development and appropriate comparisons with C punctata and C. curticei have been made. The shortest prepatent period was 17 days. The first 18 feet of the small intestine was found to harbor the majority of worms. The patent period of a single infection may last as long as 8 months.

In a study on immunity to the ruminant parasite, Trichostrongylus colubriformis, subcutaneous injection of guinea pigs with artificially exsheathed infective larvae, intraperitoneal injection with the metabolic excretions and secretions of artificially exsheathed larvae, and oral inoculations with 5,000 normal infective larvae, failed to afford any protection to the guinea pigs against subsequent oral challenge with 40,000 infective larvae.

Cooperative work at Experiment, Georgia, under a memorandum of understanding, indicated,in preliminary studies, a reduction in the number of third-stage larvae of various cattle and sheep nematodes proportional to the increase in number of viable spores of Bacillus thuringiensis var. thuringiensis Berliner.

In 1962, at Experiment, Georgia, under the auspices of the ADP Laboratory at Auburn, work on the ecological factors influencing nematode development, but using guinea pigs and rabbits for the tests, revealed a markedly lower number of adult T. colubriformis was recovered from guinea pigs infected with larvae cultured at 10°C than from three other groups of guinea pigs infected with larvae reared at 15, 25, or 32°C. The hosts infected with larvae cultured at 32°C had a much higher number of larvae than those from the other three groups. However, another test using larvae of T. axei to infect rabbits showed no difference between the number of worms recovered from the rabbits infected with larvae reared at 10 and 25°C, but a lower number of larvae was recovered from rabbits infected with larvae cultured at 32°C.

B. Effects of Mixed Helminth Infections.

In 1961, at the Regional Animal Parasite Laboratory, Auburn, Alabama, it was found that 5.5 to 6 months-old grade Jersey calves, administered from 200,000 to 700,000 infective larvae of Cooperia oncophora, developed anorexia and an enteritis during the patent period of infection. Three groups of infected calves made average total weight gains ranging from 6.5 to 20.5 pounds less than that made by the controls; however, the differences were not significant. Blood physiology was not affected by the infections.

In 1962 this work was discontinued early in the year.

C. Acquisition of Parasites from Pastures.

In 1961 at the Beltsville Parasitological Laboratory (BPL), a study was made of the development of infective larvae of gastro-intestinal parasites of cattle, their migration onto herbage, and their duration thereon following deposition of feces containing eggs of these parasites at different times of the year. In general, conditions for development, migration onto the herbage, and survival were best in early Fall and in the Spring when there was an abundance of available moisture, and they were poorest in late Fall, when it was cold, and in Midsummer when it was too dry. These findings are in agreement with earlier indications of larval development and survival obtained from the numbers of worms recovered from calves that grazed off test plots.

In 1962 at the Beltsville Parasitological Laboratory, studies were continued and indicated that rotational grazing of pastures by bovines infected with gastro-intestinal nematodes apparently did not reduce levels of parasitism or improve the performance of infected animals in an experiment carried out in 1961. Two test groups initially were artificially exposed to infection equally and simultaneously. During the experiment one of the groups grazed three 1-acre pastures rotationally. The other grazed continuously on a single 3-acre pasture. The initial and each subsequent grazing period on each smaller pasture was 2 weeks, so each 1-acre plot was vacant for 4 weeks prior to each re-grazing of it. Three cycles of rotation were completed from early July to early November, when the experiment ended and the cattle were necropsied. The worms recovered at necropsy have not yet been counted, but worm-egg counts during the test indicated no substantial difference in the worm loads of the two groups.

Initial infection with the beef measles worm (Cysticercus bovis) conferred on cattle in a recent test a strong resistance (acquired resistance) to reinfection with this larval tapeworm. The results of tests to explore the possibility that age may be a factor in initial susceptibility to this parasite suggest that adult animals, with the possible exception of ones more than 5 years old, can be at least as susceptible as calves and that individual variation in susceptibility occurs.

D. The Effect of Pasture Mixtures and Pasture Management on the Control of Internal Parasites of Cattle.

In 1961, at Experiment, Georgia, a second year feeding trial was conducted under the supervision of the Regional Animal Parasite Laboratory at Auburn. The findings reported for the first year's grazing trials were not statistically significant. The second year test was completed studying the effect of rotational grazing on different types of pasture mixtures on the level of parasitism in beef yearlings. One of 2 plots planted with temporary winter pasture was divided into 4 plots and grazed on a four-way rotational system. A third plot was also divided into four plots, each of which was planted with a different forage mixture and rotationally grazed. For the second year, the steers on the winter temporary pasture rotationally grazed harbored more worms (34,827) and exhibited a lower average daily gain (ADG) than those animals grazed continuously on the same type of forage (22,674 and 2.09 lbs.). The lower number of parasites recovered from the rotationally grazed pasture mixtures (17,149) was probably due to the excessive growth of these forages, which also reduced the ADG (1.84 lbs.), although the average stocking rate was lower than those from the other lots.

In 1962, the studies were continued at Experiment, Georgia, where the third-year test was completed in an attempt to study the effects of rotational grazing on different types of pasture mixtures on the level of parasitism in beef yearlings. One of the two lots planted with temporary pasture was divided into four plots and grazed on a four-way rotational system. A third lot was also divided into four plots, each of which was planted with a different forage mixture and rotationally grazed. The animals from the

continuously grazed pasture had less worms (15,109) and higher ADG (3.00 lb.) than those from the two rotationally grazed pastures - 41,229 worms and 2.34 lb. from the winter temporary, and 22,299 worms and 2.15 lb. from the pasture mixture. Rotation of grazing required higher stocking rate for proper pasture utilization, which may be responsible for increased parasitism.

E. Winter Coccidiosis (Bloody Scours) of Cattle.

In 1961, at the Animal Disease and Parasite Laboratory, Logan, Utah, fifteen calves were used in one experiment to investigate the possible transmission of passive immunity to coccidiosis caused by Eimeria bovis through intraperitoneal injection of concentrated serum globulin, and to observe the effects of intraperitoneal injections of sporulated oocysts, or of merozoite-mucosa emulsion on the development of clinical coccidiosis. The intraperitoneal injection of sporulated oocysts resulted in the development of mild coccidiosis sufficient to cause development of enough immunity to resist per os inoculations with 1.4 million sporulated oocysts. Intraperitoneal injections with concentrated serum globulin, merozoite-mucosa emulsion, or distilled water failed to prevent coccidiosis when the animals were given 1.4 million sporulated oocysts per os. There was no passive transfer of immunity nor alterations of the serum proteins related to any of the injections.

In another experiment sixteen yearling steer calves were used to determine how long immunity to coccidiosis persisted. Approximately 1 to 1-1.3 years after their last experimental inoculation with sporulated oocysts each of 16 calves was inoculated per os with 1.4 million sporulated oocysts. Only calves which had not previously been inoculated developed severe symptoms of coccidiosis. Severe changes in the serum protein accompanied or followed the occurrence of coccidiosis in susceptible calves. More pronounced changes in the beta and gamma globulin fractions were observed in these older calves than in the young calves. One calf, immunized about 9 months earlier and treated with sulfa drugs, developed unusually severe reductions in serum protein during the period when the susceptible calves were exhibiting severe symptoms of coccidiosis. This calf showed none of the usual symptoms seen in the other calves. This may indicate that the effects of coccidial infections are manifested in ways other than the usual diarrhea, bleeding, etc.

Thirty newly weaned calves averaging about 350 pounds each, were used to determine the effect of intraperitoneally or intramuscularly injected oocysts on the alteration of serum proteins and the development of immunity to coccidiosis. Calves injected intraperitoneally with sporulated oocysts appeared to develop resistance to per os inoculation but intramuscular injections with sporulated oocysts produced little or no immunity. Intraperitoneal and intramuscular injections with unsporulated oocysts failed to elicit the development of immunity. All of the 30 calves carried light natural infections at the time of inoculation, but per os inoculation with 300,000 sporulated oocysts produced severe effects which were sufficient to kill one calf.

In 1962 at the Logan Laboratory, the research work on winter coccidiosis was continued. No significant differences were observed in the susceptibility to coccidiosis, Eimeria bovis, infections in nursing and bucket-fed new-born calves. Uninoculated nursing control calves quickly became infected when penned with calves that had been inoculated with coccidia, but the infection was less severe. The administration of whole citrated blood intravenously to calves during the severe stage of coccidial infection, appeared to increase hemorrhage. Severe infections did not significantly alter the levels of hemoglobin, hematocrit and blood glucose, or the total serum protein.

Calves given single intraperitoneal injections with 1,000,000 sporulated oocysts of E. bovis developed clinical coccidiosis and one of eight died. Those surviving exhibited a strong immunity to reinfection. Four calves given multiple alternate day injections of 200,000 sporulated oocysts for five injections, exhibited symptoms over a longer period than calves given a single injection. One of the four calves died, but the three survivors exhibited stronger immunity to reinfection than did any of those in the other groups.

Infection was not manifested in any of another group of calves that were injected intraperitoneally with sporulated oocysts of E. bovis. This may indicate that the former injections were given into the intestinal tract instead of into the peritoneal cavity. Two calves injected intraperitoneally with x-ray irradiated oocysts that had been exposed to 60,000 r by a Westinghouse Quadrocondex machine did not develop signs of infection, or any immunity. Two calves given oral inoculations with irradiated oocysts showed a mild infection but exhibited immunity to reinfections.

There were no changes in the serum electrolytes, potassium or sodium, during prepatent or patent periods, or significant changes in the sera of those calves that survived the infections. Immediately before death, from coccidiosis, the blood serum potassium was elevated to 7-8 mEq/l while the sodium levels were reduced to 90-100 mEq/l.

In 1962, at the Montana Veterinary Research Laboratory, Agricultural Experiment Station, Bozeman, under a cooperative agreement with the USDA, research work was conducted on winter coccidiosis. Observations were made on the comparative morphology and sporulation time of Eimeria ellipsoidalis, E. bovis, E. auburnensis, E. cylindrica, E. zurnii and E. brasiliensis. An attempt was made to provide criteria for differentiation of these species, and to clarify some of the apparent inconsistencies in the taxonomy of the cattle coccidia. A progressive increase was shown in oocyst length and width from E. cylindrica through E. ellipsoidalis, E. bovis, and E. brasiliensis. Sporulation time at 20 and 30°C was also distinct.

The occurrence of coccidial strains resembling E. cylindrica either morphologically or with respect to sporulation time, but differing markedly in other respects, was demonstrated in naturally infected beef calves. It is important that valid criteria be provided for differentiating cattle coccidia, since the pathogenicity of the various species differs greatly.

F. Influence of Diet and Nutrition of Cattle on Roundworms.

In 1961, at the Beltsville Parasitological Laboratory, Beltsville, Maryland, two comparable groups of calves were fed different levels of the same diet. Half of each group was infected with equal numbers of the same kinds of gastrointestinal helminth parasites. The infected calves on the lower level of feed consumption became more heavily infected, and, relative to their respective controls were, in general, more adversely affected by the parasitism than those on the higher level. Replication is necessary before generalization is justified.

In 1962, these studies were continued at the Beltsville Parasitological Laboratory. The results showed the efficiency of feed utilization by calves on two different levels of feeding was markedly reduced by moderate infection with gastrointestinal nematodes. Efficiency was also affected by the level of feeding and was greater at the higher level. However, the reduction in efficiency caused by the parasitism was about 8 times the difference due to feeding level. Calves on the higher intake were less severely affected by the parasitism than those on the lower level. In mild infections the higher feeding level only slightly enhanced the ability of the calves to cope with the infections and efficiency of feed utilization was affected about equally by parasitism and feeding level.

G. Artificial Propagation of Protozoan Parasites.

In 1961 at the Beltsville Parasitological Laboratory, work was continued to develop a defined medium for the in vitro cultivation of Histomonas meleagridis, the causative agent of blackhead. This parasite was successfully propagated for the first time free of demonstrable bacteria in modified tissue culture media. Bacteria from the ceca of turkeys, heretofore considered important and routinely used in cultures of Histomonas, were successfully replaced by a variety of fresh hamster tissues enriched with metal ions, without loss of infectivity of the parasites. Histomonads grown in these tissue-containing media were capable of infecting young chickens when inoculated by rectum, whereas organisms grown in media enriched with bacteria from the ceca of turkeys were unable to do so.

Histomonads were grown in media devoid of cream, but containing cholesterol, cholesterol esters or a commercial steroid preparation which demonstrated for the first time that one of the growth factors for Histomonas is a steroid. It was found that certain fatty acid esters of cholesterol promote good histomonad growth, thereby indicating that another growth enhancing compound is probably a lipid.

Primary requisites to artificial cultivation of parasites are (1) freedom from contaminating organisms, and (2) an adequate supply of the infective stages of the parasite. With regard to coccidia, both these problems were solved.

Sterile suspensions of Eimeria acervulina were obtained with antibiotics. Sporocysts were released from oocysts by aseptic grinding with a mortar and pestle, and excystation of sporozoites from sporocysts was produced by treatment with sterile trypsin and chicken bile. A 1 cc suspension containing as many as 3 million motile sporozoites--more than enough--was obtained by use of trypsin and chicken or turkey bile. Excystation occurred within 5-10 minutes and 90-95 percent excysted within 1 hour.

In 1962 research workers at the Beltsville Parasitological Laboratory reported that cholesteryl palmitate and stearate were used successfully as replacements for cream in artificial cultivation of the protozoan parasite, Histomonas meleagridis. Growth factor s) for this parasite, provided by certain bacteria normally associated in the host, and grown in nutrient broth, appear to be intra-cellular. This factor(s) can be inactivated by subjecting the bacteria to temperatures above 56°C and below freezing, but can be regenerated by returning the bacteria to their normal culture temperature.

Bacteria grown in a variety of media other than nutrient broth have failed to sustain histomonad growth. A modification of a commercial tissue culture medium, known as "199", in which histomonads will grow after the addition of cream from cow's milk, was found capable of supporting histomonad growth even after being frozen for more than 2 months.

H. Host-Parasite Relationships of Coccidia.

In 1961, at the Regional Animal Parasite Laboratory, Auburn, Alabama, studies on histochemical staining revealed (a) PAS reactions were positive in E. alabamensis in oocysts in tissues and in macrogametocytes. Heavily parasitized host cells, as well as mature schizonts, ceased to be PAS positive; (b) Glycogen was found in macrogametocytes of E. alabamensis and E. zurnii, as well as in some mature schizonts of E. zurnii and E. bovis; (c) Collagen fibers and keratin were found in the outer membranes covering the macroscopic schizonts of E. bovis; (d) polysaccharides, DNA and protein were studied in macroscopic schizonts of E. auburnensis and during various times in the life cycle of E. ahsata in sheep.

An acid stain by Gray, et al, using Celestine Blue B, ferric alum, glycerol and sulfuric acid was found to be the equivalent to iron hematoxylin stain, even though only 1 minute is required for staining. Intermediate stages of coccidia in sections of cattle intestines were stained by this method.

The Lotze method of excysting oocysts of coccidia in vitro by using overnight exposure to lipase (steapsin) solution and then additional exposure to fresh bile was confirmed at the Regional Laboratory. Living sporocysts and sporozoites of E. ahsata, freed by this method, were measures.

In 5 of 6 calves inoculated with oocysts of Eimeria bukidnonensis, the prepatent period was from 11 to 16 days and the patent period from 4 to 6 days. Three calves showed signs of clinical coccidiosis.

Oocysts of Eimeria auburnensis, stored in 2% potassium dichromate solution, were still viable after 3.5 to 4 years. Schizonts of E. auburnensis, ranging up to 250 by 100u were found in the lower half of the small intestine of a calf killed on the 12th day after inoculation. Most were embedded in the mucosal layer instead of in the center of the villi as is common in E. bovis.

In 1962 studies were continued at the Auburn Laboratory. The results of several tests were (a) Schizonts were found in the middle and posterior third of the small intestine of calves killed 12 and 14 days after they had been inoculated with pure cultures of oocysts of Eimeria auburnensis. The schizonts ranged from 78u to 250u long by 78u to 150u wide. (Sample mean 92u by 139.9u). They were usually located deep in the lamina propria near the muscularis mucosae instead of in the villi where most schizonts of E. bovis are found. The schizonts of E. auburnensis resemble the previously described large microgametocytes of this species, but were distinguishable morphologically and by histochemical stains. The microgametocytes were much larger than previously reported; one measured 91u by 287.5u.

(b) Calves were killed 4, 11, 15, and 25 days after inoculating with Eimeria bukidnonensis. Studies of the tissues revealed sporozoites in sections of small intestine 48 ft. above the ileocecal valve, at 4 days. At 11 days, a young schizont was found at C + 12. Nothing was found at 15 but, at 25 days, an oocyst was located at C + 1.

(c) The following mature endogenous stages of Eimeria zurnii, E. alabamensis, and E. bovis, coccidia of cattle, were periodic acid Schiff ("PAS") positive: merozoites, microgametocytes, traces of cytoplasma and plastic granules of macrogametes and oocysts. Immature forms of the same stages were usually PAS negative.

(d) Celestin Blue B, Acid Fuchsin, and Orange G gave very good contrasting colors to various endogenous stages of Eimeria zurnii in sections of cattle intestines.

(e) In two tests on excystation of oocysts of Eimeria ahsata, a solution of chenodesoxycholic acid, a purified bile extract, did not induce liberation of sporozoites. Sodium desoxycholate, 0.5%, following lipase exposure, released 20 percent.

I. Ecology and Immunology of Lungworms.

In 1961, at the Beltsville Parasitological Laboratory, research showed that double vaccination of calves with x-irradiated larvae of the cattle lungworm usually offered some degree of protection against challenge exposure with this parasite. The immunization was accomplished with larvae exposed to 40,000 roentgens at 4 different rates - 100, 200, 400, and 1,200+ roentgens per minute. The larvae exposed at the lowest rate were reared and irradiated by a commercial firm. The rate of x-ray application did not materially affect the invasive powers of the larvae in mice and guinea pigs. However, challenge exposures produced the least pulmonary distress in calves given orally larvae

irradiated at the lowest rate. Also, these calves eliminated negligible numbers of first-stage larvae, had the least amount of lung damage at post-mortem, and no worms were grossly observed at necropsy.

In 1962, at the Beltsville Laboratory (BPL) it was found that double oral vaccination with x-irradiated cattle lungworm larvae conferred resistance to infection with this parasite on 2 of 4 pairs of test calves. At necropsy about 1 month after challenge exposure to infection with normal larvae, each of these pairs yielded fewer worms than any of 4 control calves. One calf was vaccinated with larvae irradiated at a rate of 100 r/min., the other with larvae irradiated at 1,200+ r/min. The remaining two pairs of calves were vaccinated with larvae irradiated at intermediate rates. One of these pairs was highly susceptible, the other possibly slightly resistant to infection on challenge. An adequate explanation for these differences in results is not presently available.

Oral vaccinations with larvae of a sheep lungworm appeared to be only partially successful in immunizing calves against the cattle lungworm.

J. Clinical and Physiological Aspects of Roundworms.

In 1961, at the School of Veterinary Medicine, University of California, Davis, under a cooperative agreement with the USDA, Ruelene, an organic phosphate with excellent anthelmintic properties, was found to be of greatest value when administered orally. When poured on the back, or injected intraperitoneally in cattle, anthelmintic activity was unsatisfactory. Iodine-free phenothiazine was found to have a slightly greater anthelmintic action than N.F. phenothiazine. The increased activity, however, was too small to account for the difference previously found between purified and N.F. phenothiazine.

In 1962, cooperative research at the California laboratory, showed that experimental Micellar phenothiazine is no more effective than good grade National Formula, but is much better than older commercial preparations. To obtain satisfactory anthelmintic action with phenothiazine, it is necessary to consider specific surface, purity as well as total dosage. The use of dosage recommendations based on dose area per kilogram body weight will probably give satisfactory results. The interaction of phenothiazine and certain organophosphates when utilized as anthelmintics was found to be additive rather than synergistic.

Hypoalbuminemia in heavily parasitized cattle was found to be due to reduced synthesis and only in terminal cases was an increased catabolism observed. Preliminary ferrokinetic studies in similar animals indicated that the anemia may result from bone marrow by poplasia and marrow hemolysis.

K. Investigations of Trichomonad Parasites.

In 1962, at the Regional Animal Disease Laboratory, Logan, Utah, limited research was conducted on the new project since it was necessary to acquire several cultures of Trichomonas foetus from different areas of the United States. One culture was obtained from England. Rabbits were used for antibody production since they are not known to harbor T. foetus. However, it was found that rabbits do not tolerate a long series of injections very well. Best results were obtained for separation of rabbit-serum fractions by unidimensional starch-gel electrophoresis using a voltage drop of 6 volts per centimeter at room temperature over a period of 20 - 22 hours.

In 1962, at the Utah Agricultural Experiment Station, Logan, under a cooperative agreement with the USDA, research workers isolated a pentatrichomonad from the rumen and cecum of calves. The forms of the pentatrichomonads from both locations appear to be identical.

L. Host-Parasite Relationship of Intestinal Worms Cooperia spp.

In 1962, at the Regional Animal Parasite Laboratory, Auburn, Alabama, progress was made on this, a newly instituted, project of research. It was found that calves 6 to 7½ months old, were severely affected by oral inoculation with 350,000 Cooperia pectinata infective larvae. The infected calves made an average weight gain of 3.8 pounds while the controls averaged 45.3 pounds. The clinically affected calves developed a pronounced hypoglycemia concomittant to the period of anorexia. The pathogenicity of this species is essentially as severe as that of the related form Cooperia punctata, and both species are much more pathogenic than C. oncophora.

The life history of Cooperia pectinata, an intestinal worm of cattle, is direct, requiring from 14 to 17 days to develop from the ingested infective larva to the sexually mature adult. Its rate of development is intermediate between that of C. punctata and C. oncophora. Attempts to produce hybrid nematode crosses between C. oncophora and C. pectinata were initiated. Fourth-stage female C. oncophora with fourth-stage male C. pectinata and fourth-stage male C. oncophora with fourth-stage female C. pectinata were successfully transferred to the small intestines of laparotomized helminth-free calves. Eggs in these calves' feces developed to infective larvae in culture. Photomicrographs were made of a male C. pectinata and a female C. oncophora in copula. The progeny are being studied. Chromosome preparation and staining techniques have been developed for use in this study.

A pilot study was made of the histochemistry of Obeliscoides cuniculi and of Ostertagia ostertagi and of the histochemical pathology of infection by worms of the latter species in calves. Histochemical techniques used have been Best's carmine stain, the periodic acid-Shiff reaction, the ferric mannitol technique, the Alcian blue technique, the iodine method of Nielsen, Okkels, and Stockholm (loc. cit.), and the calcium-cobalt method of Gomori for phosphomonoesterase I.

Best-positive material (glycogen) was found in the intestinal walls, ovary, and musculature of the body wall of Obeliscoides cuniculi. PA/S-positive material was detected in the cells of the digestive tract, musculature and chords of the body wall, gonads, and pseudocoelom of these worms. After treatment with malt diastase, Best-positive material is completely absent, and PA/S-positive material is absent from gonads, intestinal cells (but not the surface of the lining membrane), and musculature of body wall. Other histochemical techniques were not used with Obeliscoides.

In vitro tests using the Goodey skin penetration technique indicated that T. axei and T. colubriformis larvae were unable to effect skin penetration. Adult worms were recovered from the intestines of rabbits exposed to cutaneous applications of T. axei and T. colubriformis. The low yield of adult worms indicates that the larvae did not enter the rabbit percutaneously, but were probably ingested from the surface of the contaminated skin.

M. Anaplasmosis of Cattle.

In 1961, at the Beltsville Parasitological Laboratory, research workers reported immunity studies in cattle, using an adsorbed non-viable vaccine prepared from concentrated anaplasma-infected blood, resulted in prolonged serum antibody titers to anaplasma complement-fixing antigen. The vaccinated animals did not have appreciable immunity when exposed to low doses of infected blood.

Serologically suspicious cattle, revealed by the anaplasmosis complement-fixation test, in a large commercial herd, have been studied for anaplasmosis by inoculation tests into susceptible cattle. Two of 36 such animals (5.5%) were found to be infected. These and similar previous studies indicate the need for more research on the problem of causes of CF suspects and means for separating non-specific from specific reactions.

A series of experimental transmission trials with the Rocky Mountain wood tick (Dermacentor andersoni) have indicated that transovarian passage of the anaplasma agent in this tick is not a common finding. It was shown experimentally that infected male ticks could transmit anaplasmosis after a period of 108 days. The relative importance of these findings to actual spread of the disease in nature is still problematical, but the information obtained to date does suggest that the male tick may be responsible for considerable natural spread of tick-borne anaplasmosis.

The fluorescent antibody technic has been applied to studies on the agent of anaplasmosis. A variety of anaplasma forms, many possessing tail-like structures, have been observed in the blood of some infected animals. Filtration experiments, using plasma from acute cases of anaplasmosis, and Millipore filters of known pore size, have established the presence of an extra-cellular form of the anaplasmosis agent. Such plasma, when passed through filters of 0.65 micron and 0.3 micron average pore size, has been found to be infective for cattle.

Field trial studies at (1) Kerrville, Texas, indicate after 2 years that isolated clean offspring of carrier cattle have remained free of anaplasmosis without the use of insect control measures; (2) Evanston, Wyoming, indicate that when negative susceptible cattle are placed on tick-infested rangelands, that anaplasmosis re-infection of the animals and pastures by indigenous Rocky Mountain wood ticks has been of slight likelihood during the past year; (3) Stoneville, Mississippi, indicate that the use of an electric-eye, automatically operated walk-through sprayer, using synergized pyrethrum, can substantially reduce the losses and spread of anaplasmosis in an infected herd.

In 1962, research on anaplasmosis of cattle was continued at the Beltsville and Kerrville laboratories, with the following results:

Transmission trials using the Rocky Mountain wood tick, Dermacentor andersoni Stiles, failed to demonstrate transovarian passage of the etiological agent of anaplasmosis. Ticks of this species, collected from an infected area in Wyoming, did not transmit anaplasmosis when tested on known susceptible calves. A colony of these ticks was established and stage to stage (nymph to adult) transmission of anaplasmosis was accomplished. D. andersoni male ticks transmitted the disease after they were held for 197 days following feeding on an acute case of anaplasmosis.

The morphological variations of Anaplasma marginale Theiler, as observed in infected erythrocytes by electron microscopy and immunofluorescent methods, have been studied. The forms observed by both techniques included those with and without projections or sac-like structures. It was determined that the projection of the Anaplasma was a valid structure and not an artifact. Ticks feeding on highly parasitized blood were found to eliminate these structures in their excreta.

Serological studies were done to compare the standardized complement-fixation (CF) serum reaction of infected animals with reactions produced by serums in a capillary agglutination (CA) test. It was found that serums preserved with a low concentration of phenol as used in the CF test did not give satisfactory results in the CA test. Although there was close agreement between results of CF and CA tests, the comparative accuracy of the two testing methods was not definitely established. It was found that some CF antigens would agglutinate in the presence of immune sera from infected animals.

The causative agent of anaplasmosis was frozen by a slow-freezing technique but failed to survive prolonged cold storage even though it did survive freezing and storage at -60°C for a 24-hour period.

Experimental field control studies demonstrated a protective effect from feeding low levels of Chlortetracycline to susceptible cattle. The natural course of anaplasmosis in a dairy herd in Louisiana is being studied to evaluate the effect of the disease on milk production. Rocky Mountain wood ticks in Wyoming are being investigated as natural transmitters of anaplasmosis.

The anaplasmosis research herd, Kerrville, Texas. The objective set in 1958 for this project has been achieved by using the complement-fixation test to identify reacting animals, isolation of reactors from negative cattle by a wire fence, strict antisepsis in all handling of the animals, a minimum of arthropod control, the original reactor herd of 38 cows and a bull eventually replaced, retention of negative heifers of that herd, and by a negatively reacting herd of 46 females and a bull. Once isolation of the heifers was accomplished, there were no breaks with the disease among them.

Entomology Research Division cattle. Regular blood samples are obtained from the main herd and from cattle imported from other States for research purposes. The disease continues to spread among these cattle and clinically recognizable cases of anaplasmosis were rather frequent during the year. Since antisepsis is routinely practiced for the blood sampling, the transmission is accomplished either by arthropod vectors or some other operation.

In 1962, a project on Investigations on Anaplasmosis, Piroplasmosis and Babesiellosis of Cattle was initiated under a PL 480 grant to the School of Veterinary, Montevideo, Uruguay. No report on progress was submitted for the quarter ending June 30, 1962.

In 1962, a project on Investigations on the Pathogenesis of Lesions Produced by the local Leech, Limmatis nilotica, was initiated under a PL 480 grant to the Hadassah Medical School, Hebrew University of Jerusalem, Israel. No report on progress has been made.

PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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AREA NO. 11 - PARASITES AND PARASITIC DISEASES OF SWINE

Problem. Parasitic diseases have been estimated to cost the swine industry of the United States at least $200 million annually. These diseases for the most part are cosmopolitan. Subclinical infections are the most frequent type and the most costly, yet they are generally so difficult to recognize that they often are overlooked entirely. Diagnosis is difficult, and successful treatments for many of these parasitisms are not available. Moreover, management practices to avoid the spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling, or eradicating parasitic diseases so as to provide for healthy swine, insure adequate supplies of parasite-free pork for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA PROGRAM

The Department has a continuing long-term program involving parasitologists, veterinarians, biochemists, microbiologists, and pathologists engaged in basic and applied research in this problem area. Research is being conducted on the following diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 4.7 professional man years. This effort is divided among sub-headings as follows:

The role of parasites in the economy of swine production 1.2 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, and at the Division's laboratory at Tifton, Georgia, through informal cooperation with the Georgia Coastal Plain Experiment Station.

Bionomics and pathogenicity of the swine whipworm 0.5 at the Beltsville Parasitological Laboratory.

Swine kidney worms 2.1 at Tifton, Georgia, the Beltsville Parasitological Laboratory, and under cooperative agreement with the North Carolina Agricultural Experiment Station at Raleigh.

Investigations of Trichinella spiralis 0.5 at the Beltsville Parasitological Laboratory.

Effect of anthelmintic treatment on rate of gain 0.3 at Tifton, Georgia.

Pathogenic role of the intestinal roundworm 0.1 under a cooperative agreement with the Nebraska Agricultural Experiment Station at Lincoln.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 3.9 professional man years divided among sub-headings as follows: effect of anthelmintic treatment on rate of gain 0.4; swine kidney worms 0.5; pathogenic role of the intestinal roundworm 1.0; biology and pathology of the swine whipworm and other parasites 2.0. Work in the North Central Region deals with anthelmintic treatment, roundworms, and biology and pathology of the swine whipworm and other parasites. Work in the Southern Region deals with anthelmintic treatment, roundworms, and kidney worms. The Western Region is carrying out work on anthelmintic treatment.

Industry and other organizations. Several chemical companies are engaged in the formulation of compounds and explorations for chemicals that may be used safely in the treatment of swine for elimination of worms. Generally, these companies have their own facilities, including laboratories, barns, and other structures containing pens for experimental animals, and in some cases, pastures. The work of these companies and the results, expenditures, and related matters are ordinarily confidential, since they involve eventually saleable products. Estimated annual expenditures are equivalent to approximately 15 professional man years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. The role of parasites in the economy of swine production.

In 1961, at the Beltsville Parasitological Laboratory, studies of the lipid content of helminth parasites were initiated in an attempt to learn more about the fat metabolism of helminths. This type of investigation was only made possible by the development of new techniques and information obtained was useful in increasing our understanding of parasitic disease and of developing new methods for the control of parasites by chemical and biological methods. Four species of helminths and one species of lice were analyzed in this preliminary study and several higher carbon-number fatty acids were found to be present. The lipids in the helminths (swine nodular worms) were similar to those found in the intestinal mucus in which they live. The presence of odd-carbon-number fatty acids in these parasites indicated that the worms may feed on bacteria. The lipids of the louse were somewhat different from those found in the blood of the host. This finding indicated that the lice either metabolize the lipids or concentrate them.

One of the nodular worms of swine, Oesophagostomum quadrispinulatum, was found to consist of possibly three different strains. Two of these have a 20-day prepatent period, whereas the other has one of 50 days. One of the 20-day strains has tails shorter than the other 20-day strain. The tails of the latter and of the 50-day strain are relatively long. There is some evidence that the short-tailed form can be used for genetic studies.

The search for cheaper and more satisfactory materials than animal charcoal for use in making cultures of round worm eggs in swine feces resulted in the finding that peat moss, sphagnum moss, vermiculite, sawdust, sand, and topsoil all produce satisfactory results. Peat moss was considered the most satisfactory material for culturing eggs of the nodular worms and red stomach worms of swine. The relative quantities of water, feces, and the above-named materials and the thoroughness with which the ingredients were mixed were found to be quite important in obtaining large yields of infective larvae. The quantity of the various ingredients vary with the amount of moisture in the fecal sample. However, satisfactory results were obtained when 40 to 80 ml. of water, 20 grams of peat moss to 595 grams of sand were mixed with 100 grams of fecal material.

Studies indicated that adult nodular worms can become established in another swine host after being transferred to it by means of an enema tube via the anus. Seven pigs were successfully infected by this means.

Infective larvae of swine nodular worms were observed to be capable of infecting susceptible pigs after being stored at ordinary refrigerator temperatures (4°C.) for 575 days.

In 1962 further investigation of the lipids in the tissues of the swine nodular worm, Oesophagostomum quadrispinulatum, demonstrated that all five kinds of phospholipids were present - non-choline phospholipids, inositol phosphatide, lecithin, sphingomyelin, and lysolecithin. In addition, studies on two intestinal nematodes of chickens, Ascaridia galli and Heterakis gallinarum, the pork muscle worm, Trichinella spiralis, and one free-living nematode, Ditylenchus myceliophagus, disclosed the presence of 28, 33, 30, and 28 fatty acids, respectively. All of the last-mentioned compounds were straight chain fatty acids, whereas 10 of the 33 fatty acids reported from the swine nodular worm in 1961 had side chains. The three parasitic nematodes had a complete series of saturated straight chain fatty acids ranging from 9 to 20 carbon atoms. Fatty acids having 9, 13, 17, and 19 carbon atoms were missing from the free-living nematode. From 44 to 58 percent of the fatty acids in the parasitic worms were unsaturated, whereas 65 percent of those in the free-living nematode were in this group. The fatty acid having 16 carbon atoms and two double bonds was prevalent in the nematodes from the chicken. The most abundant fatty acids in the free-living nematode were those with 18 and 22 carbon atoms having one unsaturated bond. The significance of these findings is yet to be determined.

In 1962 at Tifton, Georgia, the effect of Strongyloides ransomi on the rate of gain of pigs of different ages and on different levels of nutrition was less than the effect of the age of the pigs and the levels of nutrition as compared with inoculated and non-inoculated, full-fed and limited-fed, and 80 lb. and 40 lb. groups. This was attributed mainly to the late establishment of infections in the inoculated groups of pigs.

Strongyloides ransomi infections in baby pigs may cause death or serious loss in thriftiness. Diagnosis of natural infections and observations of experimental inoculations given to pregnant sows indicate the possibility of prenatal infections of this species as a common occurrence.

Diagnosis of field cases of parasitism again showed Strongyloides ransomi to be the most prevalent parasite to cause death and unthriftiness in baby pigs. Ostertagia ostertagi was the most prevalent species in clinical cases of parasitism in cattle and was the cause of the loss of 6 brood cattle of 3 to 6 years of age.

B. Bionomics and pathogenicity of the swine whipworm.

In 1961, at the Beltsville Parasitological Laboratory, observations on the period during which swine whipworm eggs, placed on the surface or buried in soil up to 8 inches, remain infective to pigs in the vicinity of Beltsville were continued. Eggs deposited during the summer months survived 4 and 1/2 years on the surface of the plots, and at depths of 4 and 8 inches. Eggs deposited during the winter months have survived 2 years on the surface of the soil and at a depth of 4 inches. However, samples from the surface of plots contaminated in June 1956, did not produce infections in pigs when administered to them in April 1961. The infectivity of the buried eggs was not tested at that time. These results demonstrated that the swine whipworm egg remains infective to pigs for as long as 4 and 1/2 years in the absence of recontamination, whether on the surface of the soil or buried as deep as 8 inches.

Several attempts were made to find and describe the early stages of the parasitic portion of the life cycle of the swine whipworm, which up to the present time have not been described. Pigs were fed 50,000 embryonated eggs of Trichuris suis and were necropsied on the 7th, 9th, 13th, 21st, and 23rd day after infection. In stained sections of the caecum and large intestine, portions of nematode larvae were found deeply embedded in the crypts of the mucosa on the 9th day, and on the surface of the mucosa on the 13th day. A few first-stage larvae were recovered from the lumen of the large intestine and cecum on the 23rd day, and the first- and second-stage larvae on the 21st day.

C. Swine kidney worms.

In 1961, at Tifton, Georgia, the program for the eradication of kidney worms from an experimentally infected area at the Georgia Coastal Plains Experiment Station was completed. The program was based on limiting the breeding herd to young gilts and disposing of them after weaning their first litter. The incidence of kidney worm infections in pigs on contaminated lots was reduced from 88 percent (Spring, 1959) to 34 percent (Fall, 1959) to 0 percent in the Spring of 1960. A similar experiment is under way on a farm where a heavy natural infection of kidney worms was prevalent.

In 1962, at Tifton, Georgia, it was reported that the incidence of kidney worm infection in pigs on the experimental farm, raised from gilts in a kidney worm contaminated lot, was 93 percent for the spring group of 1961 pigs and 50 percent for the fall group. The incidence in pigs farrowed in an adjacent lot from gilts free of kidney worms was zero for both the spring and fall groups.

In 1961, in work performed under a cooperative agreement with the North Carolina Agricultural Experiment Station, Raleigh, there were indications that prenatal infection with swine kidney worms might occur if sows were exposed to kidney worm larvae during pregnancy. In pigs fed kidney worm larvae at 8 weeks of age, eosinophilia appeared at the second week and reached a peak at the seventh to ninth weeks, and continued at a high level through the sixteenth week. The sedimentation rate increased at the fifth week and continued at a high rate through the sixteenth week. A series of compounds, including the organic phosphate group, failed to stop ova production in kidney worm-infected sows when administered orally or by inoculation within a safe level of medication.

In 1962, at the North Carolina Station, in studies to substantiate prenatal infection, six gilts reared colostrum-free in air-lock isolation units, were given 500 infective S. dentatus larvae weekly. These doses began at first heat and will continue throughout gestation. The gilts were bred at second heat period. Arrangements have been made to obtain colostrum- and parasite-free pigs to be penned with the offspring of these gilts for positive controls. The pigs from the gilts will be weaned at 8 weeks of age and the sows autopsied to determine status of kidney worm infection in the dams. Blood samples will be taken from the pigs for cytological and immunophoretic studies. Postmortem examinations will be made of pigs from different litters at 6 and 12 months of age.

Sows were purchased from a local abattoir following demonstration of ova in urine. They were confined in a quonset hut with concrete floors that were scrubbed daily to remove source of reinfection. The ova in the urine of these sows were used as a source of infection for experimental animals. A large Duroc sow is passing viable ova in urine after three years. There was a slight drop in concentration of ova after 30 months. A Poland China sow is producing large numbers of ova after confinement for 16 months.

Several compounds have been evaluated as possible anthelmintics against the swine kidney worm. Naturally infected sows were purchased following the demonstration of ova in the urine. The compound under investigation was given orally or as an injection. Examinations were made of the urine to determine influence on ova production. Compounds evaluated included Bayer 13/59, Bayer 21/199, Bayer 29493, Ruelene, and Tennecetin. No satisfactory compound was found that decreased ova production within a safe level of medication.

Substantiation of prenatal infection would aid in the understanding of the life cycle of the kidney worm. This phenomenon could shorten the prepatent period by 2 to 3 months and help explain the presence of sexually mature parasites in young pigs. A patent infection in sows for as long as 3 years would greatly intensify the problem of control, as these sows could contaminate ground and provide source of infection for several litters of pigs.

D. Investigations of Trichinella spiralis.

In 1961 at the Beltsville Parasitological Laboratory, it was reported that, although the standard digestion technique is considered to be a very dependable method for detecting trichinae in meat samples, it is time-consuming and requires relatively expensive and elaborate apparatus. A less complicated and more rapid method was therefore developed in which the psoas muscle of pigs infected with trichinae was blendorized, passed through a 100-mesh screen, and the sediment examined with a dissecting microscope. In this test trichinae were detected in a 20-gram sample of the psoas muscle of an 80-pound pig that had received 80 cysts of Trichinella spiralis. The digestion technique was then employed to determine the distribution of trichinae in 200-pound pigs that had received 10 to 100 cysts. Muscles of the neck, tongue, cheek, shoulder, loin, ham, diaphragm and chest wall, and the psoas muscle, were examined. More larvae per gram of tissue were usually found in the cheek, diaphragm, and tongue than in the other tissues, but no consistent pattern of distribution was noted.

In 1962 no work was reported for this project.

E. Effect of anthelmintic treatment on rate of gain.

In 1961 and 1962, at Tifton, Georgia, the effect of anthelmintic treatment (sodium fluoride against Ascaris suum) on rate of gain when administered to parasitized pigs of different ages and on different levels of nutrition was less than the effect of the age of the pigs and the levels of nutrition. This was attributed mainly to the low rate of parasite infection.

F. Pathogenic role of the intestinal roundworm.

In 1961, in research studies under a cooperative agreement with the Nebraska Agricultural Experiment Station, Lincoln, trypsin and chymotrypsin inhibitors were extracted from the body fluid and body wall of adult ascarids. Six-month-old pigs were as readily infected with ascarids as were five-week-old pigs. Liver lesions caused by migrating Ascaris healed within 21 days. Ascaris larvae migrated readily through the liver and lungs of pigs which had been given two previous exposures of infective Ascaris eggs. Thiabendazole, at 0.25 percent of a milk diet, greatly reduced the number of Ascaris larvae within the liver and lungs of 4 pigs. Ascaris infections have occurred in all of 30 herds of repopulated specific pathogen-free (SPF) pigs.

In 1962 at the Nebraska Station, subcutaneously injected thiabendazole effectively stopped migrating Ascaris suum. Hygromycin B, and a cadmium formulation effectively removed adult ascarids from swine. A N-Benyl derivative of Hygromycin was ineffective. A special formulation of an organic phosphate, 2,2 Dichlorovinyl dimethyl phosphate, was highly efficacious against A. suum and Trichuris suis in swine. Guinea pigs demonstrated a good immunity to A. suum when injected with infective eggs and specific protein components of the adult worms.

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PUBLICATIONS REPORTING RESULTS OF USDA AND COOPERATIVE RESEARCH

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AREA NO. 12 - PARASITES AND PARASITIC DISEASES OF SHEEP AND GOATS

Problem. The cost of parasitic diseases to the sheep and goat industry of the United States is estimated to be in excess of $45 million, annually. Disorders caused by parasites are ubiquitous, generally insidious and often overlooked entirely. Diagnosis is difficult, and successful treatments for many of these diseases are not available. Moreover, management practices to avoid spread of parasitisms and to control them are often ineffectual. The problem is to develop, through a planned, balanced program of basic and applied research, knowledge for preventing, controlling or eradicating parasitic diseases so as to provide for healthy animals, insure adequate supplies of high quality lamb for an expanding population, avoid or minimize economic losses caused by these diseases, and thereby contribute to a prosperous agriculture, a sound national economy, a high standard of living, and a healthy population.

USDA PROGRAM

The Department has a continuous long-term program involving biochemists, parasitologists, and veterinarians engaged in both basic studies and the application of known principles to the solution of parasites and parasitic diseases of sheep and goats. Research is being conducted on these diseases at the designated locations.

The Federal scientific effort devoted to research in this area totals 8.3 professional man-years. This effort is divided among sub-headings as follows:

Lungworms 1.0 at the Beltsville Parasitological Laboratory.

Bionomics of Coccidial Parasites 2.0 at the Beltsville Parasitological Laboratory.

Effects of Helminth Infections on Serum Proteins 0.5 at the Beltsville Parasitological Laboratory.

Gastrointestinal Nematodes 2.1 at the Beltsville Parasitological Laboratory, and under a cooperative agreement with the Kentucky Agricultural Experiment Station at Lexington.

Helminth and Protozoan Parasitism in the South 1.5 at the Regional Animal Disease Research Laboratory, Auburn, Alabama, and through informal cooperation with the Mississippi Agricultural Experiment Station, State College.

Biology, Pathogenesis, and Control of Helminth Parasites of Sheep in the Southwest 1.0 at the University Park, New Mexico, field station, and through informal cooperation with the New Mexico Agricultural Experiment Station, at University Park.

Biology of the Liver Fluke 0.1 under cooperative agreement with the Montana Agricultural Experiment Station, Bozeman.

Effect of Intestinal Roundworms on Metabolism 0.1 under cooperative agreement with the North Dakota Agricultural Experiment Station, Fargo.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported 5.6 professional man-years devoted to this research. Studies are aimed at locating areas of parasitism with the lungworm and developing information on the habits of the parasite which will be useful in its control. Research is directed toward means for controlling coccidial parasites. Four western States have cooperative studies through regional research project W-35, Nematode Parasites of Ruminants to clarify some of the major problems caused by gastrointestinal nematodes. Management procedures are being developed based on critical observations of parasite incidence under different systems of flock management. Genetic resistance is being evaluated with the possibility that some breeds, or lines within breeds, may be more resistant to certain parasites. Improved methods are being developed for diagnosing infections with specific parasite species. Parasitologists are seeking to identify snails which serve as intermediate hosts of liver flukes and are determining factors concerning the ecology of these snails which may provide a means for breaking the life cycle of the fluke. Enzootic areas of fluke infestation are being located and methods of elimination evaluated. Studies at Nevada are aimed at measuring precisely the damage caused by flukes and how this damage is produced in order that scientifically sound countermeasures can be evolved. Prevention through immunization is under study. Existing control measures are being applied to determine their effectiveness under conditions found within the State.

Industry and other organizations are engaged in the formulation of compounds and explorations for chemicals that may be used safely as parasiticides. Generally, these companies have their own facilities, including laboratories, barns, and other structures containing pens for experimental animals, and in some cases pastures. The work of these companies and the results, expenditures and related matters are ordinarily confidential, since they involve eventually saleable products. Estimated annual expenditures are equivalent to approximately 20 professional man-years.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Lungworms.

In 1961, at the Beltsville Parasitological Laboratory, Beltsville, Maryland, an experiment to determine the earliest age at which larvae of the thread lungworm, Dictyocaulus filaria, can infect sheep demonstrated that larvae only 100 hours after passage from infected sheep may produce fatal infections when administered to lambs.

In 1962, at this Laboratory, lambs and kids that had recovered from thread lungworm infections were still strongly immune to reinfection up to 15-1/2 months after recovery, even when challenged with as many as 60,000 larvae. Comparable susceptible animals developed severe infections when given the same larval dosages. Serum protein changes and eosinophilia in the immune animals indicated that the challenge exposures had stimulated the immune responses of the host even when no other evidence of exposure was noted. Growth and sexual development of worms were definitely inhibited in immune animals and the immune reactions appeared to be active against all stages of migration and development. Resistance against lungworm infection developed in the lungs of kids in which the mesenteric lymph nodes were by-passed by infecting fourth-stage larvae into the jugular vein. The presence of circulating antibodies was indicated by the deactivation of fourth-stage larvae by immune serum and the appearance of a diffuse precipitate in the vicinity of the worm.

A moderate immunity to sheep lungworm was apparently produced by two exposures of lambs to infective larvae of the cattle lungworm. Reduced larval production, reduced worm burdens, inhibited worm development, and reduced lung pathology were found in "immunized" animals.

B. Bionomics of Coccidial Parasites.

In 1961, investigations at the Beltsville Parasitological Laboratory showed that suckling lambs may be as ready prey to coccidiosis as weaned lambs. The studies involved 10-week-old suckling lambs that were experimentally fed moderate to large numbers of oocysts of two or three species of coccidia from sheep. This study indicates that, contrary to a rather widespread belief, late weaning of lambs may be of little benefit from the standpoint of susceptibility to coccidiosis, all things being equal.

Studies indicated that at least some species of coccidia parasitic in sheep, although able to infect goats, complete only a portion of the life cycle in these latter animals, which are but little affected by the infection. These studies indicated that within a species, there are physiologic strains, some of which are adapted to sheep and others to goats. In this study, stages of coccidia normally occurring in the intestinal epithelium, and not known to occur elsewhere in the body of hosts, were found for the first time in the mesenteric lymph nodes of a sheep and a goat.

Oocysts of coccidia swallowed by rodents may respond to conditions therein by partial, but not complete, excystation. It was not determined whether such oocysts are infective to sheep and may, therefore, be disseminated by rodents, but in light of similar observations previously made on eggs of certain nematodes, the possibility is considered strong.

That a relationship can exist between an imperfect condition of wool and experimental coccidiosis was confirmed this year. About one month after infection, when coccidiosis had run its course, breaking of wool and loss thereof occurred over significantly large areas of the bodies of the affected animals.

Oocysts of ovine coccidia that had been stored in water for 5 years, were determined to be viable through the application of an in vitro test.

Studies on excystation of coccidial oocysts in their hosts, and in vitro, the latter conducted by the enzyme-bile technique mentioned last year, presented evidence that the mechanism of excystation, and infection, of sheep coccidia and avian coccidia, are essentially similar. In these studies evidence was obtained that excystment of the parasites follows a change in the physiology of the parasites from that of the quiescent stage outside the host, to one of activity for parasitism in the host; in sheep coccidia and in a turkey coccidium, the activity of excystment was renewed almost immediately when the parasites were warmed on the warm stage of the microscope after they had been interrupted in the excystment process by placing them for over a week in the refrigerator.

The studies revealed, moreover, that sheep coccidia need an incubation period of about 5 hours to start the physical excystment process in vitro and that the following conditions will serve the purpose - (1) incubate for 5 hours in pure bile; (2) incubate for 5 hours in saliva or steapsin solution, then add bile; and (3) incubate for 3 hours in distilled water, then for 2 hours in the enzyme solution, and then place in bile.

In 1962, at the Beltsville Parasitological Laboratory, it was found that enzymes of plant origin influence hatching (excystation) of oocysts of sheep coccidia. The enzymes found by in vitro tests to promote excystation, include alpha and beta amylase, Takamine pectinase and cellulase, and an amylase and a protease derived from fungi. Although not known to be the case, it is possible that active enzymes of plant origin may occur in the digestive tract of sheep and thereby influence infection of these animals with coccidia.

Hatching (excystation) of oocysts of coccidial parasites of sheep occurs only after a period of physiological development of the invasive bodies (sporozoites) contained therein. This development, permanent in nature and capable of being interrupted and then resumed, can be initiated by exposure of the ripened (fully developed) oocysts to subcutaneous fluids of the host (5 hours) or to digestive enzymes of the host for about 17 hours.

C. Effects of Helminth Infections on Serum Proteins.

In 1961, at the Beltsville Parasitological Laboratory, a study was conducted in cooperation with the Animal Husbandry Research Division on the changes in the blood serum proteins of 4 groups of 5 cross-bred ram lambs each, which were subjected, respectively, to minimal, moderate, and heavy exposures to parasitic infection. These lambs were part of the same experimental bands discussed in the report on gastrointestinal nematodes. Serum protein studies verified the parasitological data acquired in the aforementioned experiment that severe helminthic infection was prevented from occurring in these lambs by a combination of good management practices and timely use of proper medication. Albumin to globulin (A/G) ratios and serum globulin percentages

fluctuated but did not depart markedly from the normal range in any of the 4 management bands. Gamma globulin percentages did not change markedly in any of the groups, and were relatively comparable. However, the beta globulin percentage varied more noticeably than the gamma globulin percentage, but were relatively minor and within the normal range. Another indication that parasitism did not attain gross proportions in these animals was the absence of significant quantitative changes in the total serum proteins (TSP). In all 4 bands the average TSP was higher at the end of the study than at its inception. The results of this study extend and confirm the conclusions derived from prior studies in this series in that the degree of change in serum proteins of lambs apparently depends to some extent on the type and severity of parasitism and possibly on the breed of the host animal.

In 1962, at this Laboratory, no work was done on this project because of reductions in personnel and in numbers of experimental animals. The project leader went to Australia on a Fulbright Grant to study sheep parasites for one year. The reduction in numbers of experimental animals was occasioned by having to dispose of the entire sheep flock prior to moving to the area formerly occupied by the Animal Disease Station at the Agricultural Research Center, Beltsville, Maryland.

D. Gastrointestinal Nematodes.

In 1961, at the Beltsville Parasitological Laboratory, in cooperation with the Animal Husbandry Research Division, and the Antiparasitic Investigations Research Group of this Division, a second-year study was conducted to determine the effect of various types of management, including anthelmintic treatments, on parasitism in lambs. Four bands of 75 lambs each were studied. Band 1 was quartered on a dry lot only and fed pellets and alfalfa hay: Band 2 was moved to clean pastures periodically: Bands 3 and 4 were moved bi-weekly to previously grazed, or contaminated pastures. All lambs were on continuous phenothiazine-salt prophylaxis. Bands 2 and 3 also received therapeutic medication with N.F. phenothiazine, while Band 4 received the purified form of the drug, after the appearance of clinical parasitism. Conclusions resulting from the previous year's study, such as the earlier institution of anthelmintic treatments and/or using a purified form of phenothiazine, were implemented successfully in producing greater weight gains and reducing anemia and fatalities, particularly from haemonchosis, in the lambs under surveillance. Data obtained from the periodic examination of the feces for parasite eggs of 20 representative ram lambs of each band, and from necropsy of 40 lambs during the course of the study, indicated that (1) the lambs on dry lot remained essentially parasite-free, and made excellent weight gains; (2) the lambs on clean pastures gradually developed clinical parasitism during the summer, but neither the weight gains nor the hematocrits were appreciably affected; (3) Band 3 lambs on contaminated pastures and treated with N.F. phenothiazine developed haemonchosis and showed anemia and reduced weight gains early in July, and (4) Band 4 lambs, also on contaminated pastures but treated with purified phenothiazine maintained higher blood levels and greater weight gains than the lambs of Band 3. Worm counts at necropsy showed lambs of Band 4 had fewer worms, especially Haemonchus contortus, than

Band 3. These counts revealed that marked Haemonchus infections occurred in Band 3 in June, August, and September, decreasing somewhat after each therapeutic treatment with N.F. phenothiazine, whereas, treatments with purified phenothiazine kept Haemonchus under continuous control in Band 4. Although lambs of Bands 2, 3, and 4 harbored moderate numbers of Strongyloides papillosus, strongyloidiasis was not an important factor as in the previous year. Other than the two species of worms already mentioned, small numbers of Cooperia, Trichostrongylus, Nematodirus, Trichuris, and Oesophagostomum were recovered. Deaths from parasitism occurred only in Band 4, and then amounted to less than 3 percent. The mortality figures were much lower than those of the preceding year, demonstrating the effectiveness of proper management and the judicious use of medication.

In 1962, at the Beltsville Parasitological Laboratory, the work was continued for a third-year study on the effects of pasture management and chemotherapy in relation to parasitism, and confirmed the results of previous years. Band 1 lambs raised on dry lot and fed green chop remained essentially parasite-free and showed no effects of parasitism. Band 2 lambs raised on clean pastures gradually developed clinical parasitism but none was severely affected. Band 3 lambs, on contaminated pastures, developed clinical parasitism relatively early in the grazing season which was not adequately controlled by 2 treatments with N.F. phenothiazine, but which was reduced to a lower level by 2 subsequent treatments with purified phenothiazine. Band 4 lambs on contaminated pastures. developed clinical parasitism and received 3 treatments with purified phenothiazine. These treatments reduced the effects of parasitism but were not as effective as the 4 treatments in Band 3. Individual animals in both bands 3 and 4 became seriously anemic but only one death resulted from haemonchosis; this death occurred in Band 3. Parasites other than Haemonchus contortus were present in insignificant numbers and apparently had little clinical effect. Overall, excellent control of clinical parasitism was achieved by the management and therapeutic techniques employed.

In 1961, at Lexington, Kentucky, under a cooperative agreement with the Agricultural Experiment Station, and in informal cooperation with Southern Regional Research Project S-21, "Gastrointestinal Parasites of Ruminants," it was found that the second year's observations on pure infections of Haemonchus contortus under pasture conditions indicated that the free-choice consumption of phenothiazine-salt (1:9) was more effective against control strain A than the resistant strain B. The natural transmission of H. contortus to the lambs in the untreated groups on these pastures started between the middle of May and the first of June in 1960. The epidemiology also showed a winter carry-over of immature stages or agamous adults in ewes.

In a field test during the 1960 grazing season a series of Ruelene drenches at 3-week intervals were more effective than the same number of phenothiazine drenches in controlling the gastro-intestinal parasites of lambs on pasture. Although one of 10 lambs treated with Ruelene died during the course of the study, the Ruelene-treated lambs average daily gain was slightly better than the phenothiazine-treated lambs. A similar group of lambs kept under dry-lot conditions and treated with 4 Ruelene drenches at 3-week intervals, showed poor weight gains and 2 of 5 animals died.

A series of tests with a purified phenothiazine against experimental infections of resistant strain B H. contortus in lambs resulted in a higher (but not statistically significant) average removal efficacy than 2 N.F. Green phenothiazines.

In 2 field tests, single oral doses of MK-360, Ruelene, and phenothiazine ranked in this order in efficacy in reducing post-treatment egg counts in feces.

Rendering rats, which are naturally refractive to T. axei, visibly vitamin A deficient, did not make them susceptible to this worm.

Continued efforts to cultivate T. axei in vitro were unsuccessful.

In 1962, at the Kentucky Agricultural Experiment Station, and in informal cooperation with Regional Project S-21, it was found that in the third year's observations on pure infections of Haemonchus contortus under pasture conditions the free choice consumption of phenothiazine-salt (1:9) continued to be much more effective against control strain A than the phenothiazine-resistant strain B.

Laboratory tests on experimental infections of strain B H. contortus comparing removal efficacies of single doses at .1 gm/lb. of two N.F. green on one purified phenothiazine product resulted in no significant difference of activity. Likewise, laboratory tests on experimental infections of strain B H. contortus comparing suppression of egg production and inhibition of larval development of small (.1 gm and .25 gm) daily doses of regular N.F. green, microfine, and microfine-purified phenothiazine products resulted in no significant differences among the six preparations tested.

In a controlled test of anthelmintic activity in lambs thiabendazole at 50 mg/kg was more completely effective against a greater number of species than the organic phosphate Famophos (Cl 38,023) at 100 mg/kg. The latter was characterized by activity shortcomings against Strongyloides, Nematodirus and Oesophagostomum and immature worms. Neither compound was active on Trichuris.

Field tests on the anthelmintic activity of organic phosphates following pour-on administration in cattle revealed activity of SD3562, Famophos, and Ruelene in reducing post-treatment EPG, whereas Neguvon and Tiguvon were devoid of action.

Rats visibly vitamin A-deficient, were not susceptible to Trichostrongylus axei.

Mongolian gerbils were successfully infected with the animal pathogen T. axei. The technical and economic advantages of this laboratory host-parasite combination are numerous.

E. Helminth and Protozoan Parasitism in the South.

In 1961, at the Regional Animal Disease Research Laboratory at Auburn, Alabama, experimental lambs showed some resistance to reinfections with coccidia when given repeated doses of sporulated oocysts mixed in their feed. This resistance was more apparent in the 3rd and 4th inoculations and was demonstrated for Eimeria faurei, E. crandallis, E. arloingi, and E. ninakohlyakimovae. The resistance to reinfection appears to be species specific. Only one species of coccidia, E. ahsata, produced a strong immunity to reinfection after a moderate to heavy infection.

A preliminary investigation on the life cycle of Eimeria ahsata, a coccidian parasite that was recently found to be very pathogenic to lambs, revealed (a) at 2 days after inoculation sporozoites were found in epithelial cells of the lower small intestine; (b) young schizonts were located in the duodenum and jejunum on the 4th and 7th day; (c) at 14 days schizonts were found throughout the small intestine with as many as 4 in the lacteals of one villus. They ranged up to 220u in size and some had very thick coats; (d) macroscopic schizonts were still present at 17 days, at which time gametocytes and developing oocysts were noted in the cecum and colon.

In 1962 at the Regional Laboratory, mild clinical signs were noted for the first time accompanying a heavy infection of Eimeria crandallis in a 2-month-old lamb. The oocysts of Eimeria crandallis were found in the epithelial cells covering the ends of villi of a lamb, causing the villi to have a distinctive, tiny circle which could be of value in diagnostic examinations. Large numbers of oocysts are not necessary to infect or reinfect lambs with Eimeria arloingi. Lambs receiving approximately 400 sporulated oocysts per day of this species for 20 days showed heavier infections than those receiving 800 per day for 10 days and another receiving a single dose of 8000 oocysts.

Additional information was obtained on the endogenous stages of the life cycle of Eimeria ahsata, a highly pathogenic coccidian in lambs. Sporozoites were found in the upper small intestine. Immature schizonts were in the lower part of the small intestine. Young schizonts were covered with a very thick wall of host material that was fibrous, with the cilia-like strands radiating outward. Microgametocytes, macrogametocytes, and schizonts were located, measured, and photographed. The largest schizont, at 15 days, measured 162.5u by 265u. As few as 16,000 oocysts of Eimeria ahsata caused massive infections of a young lamb, with very few indications of clinical coccidiosis. When 31,000 oocysts were given to two others, the infections caused clinical coccidiosis in both and the death of one lamb. Many oocysts expelled during the last three to eight days of these massive infections proved to be malformed, delicate "duds" that distorted easily during manipulations.

In 1961, through informal cooperation with the Mississippi Agricultural Experiment Station and the Southern Regional Animal Disease Research Laboratory, a study comparing the degree of parasitism in early and late lambs during 1960 showed the same general trend as in previous years, namely, that at any given age, late (February-born) lambs harbor more parasites than early (November-born) lambs of the same age.

In 1962 these same research laboratories reported previous work showed that late lambs of a given age would harbor more worms than early lambs of the same age. This year, lambs grazing pastures together had comparable numbers of worms regardless of age, at least after 120 days of age, indicating that time of year for grazing is more important than age of the host in the acquisition of parasites by sheep.

The research at Auburn, Alabama, and State College, Mississippi, on Helminth and Protozoan Parasitism in the South, was coordinated with Southern Regional Project, S-21 on "Gastrointestinal Parasites of Ruminants."

F. Biology, Pathogenesis, and Control of Helminth Parasites.

In 1961, at the University Park, New Mexico, Field Station, with informal cooperation with the New Mexico Agricultural Experiment Station, and in informal cooperation and coordination with the Western Regional Project W-35, Nematode Parasites of Ruminants, the following research was reported on the life histories, biology, and pathogenesis and control of certain helminth parasites of sheep in the Southwest:

Immunization of Sheep against Haemonchosis: In a controlled test with 18 worm-free lambs of similar age, breeding, and sex, it was demonstrated that a strain of Haemonchus from pronghorn antelope was significantly less pathogenic than was a strain which originated in domestic sheep. Criteria used to evaluate pathogenicity were weight gains, feed consumption, hemoglobin levels, packed cell volumes, and worm egg counts. Utilizing these same criteria and 12 of the 18 lambs used previously, it was further demonstrated that inoculation with the relatively non-pathogenic antelope strain gave the lambs a significant degree of protection against haemonchosis caused by the sheep strain.

Life History of the Fringed Tapeworm of Sheep: These studies were expanded and intensified by collecting, identifying, and culturing psocid material from new sheep range locations in the Southwest, by experimenting with new diets and substrate modifications in an effort to improve the survival time of the insects in culture, and by modifying the diet of test lambs to make them more susceptible to experimental infection. Attempts made to date to infect lambs experimentally have been unsuccessful.

Failure of Moniezia to Develop in Psocids: Five cultures of various species of psocids were established and the contained insects were exposed to the eggs of Moniezia. The subsequent dissection of 41 psocids from these cultures failed to reveal any developmental stages of cestodes. There was no evidence, therefore, that these insects play any part in the transmission of Moniezia to sheep.

Investigations of the Life Histories of Elaeophora schneideri, Nematodirus lanceolatus, and Nematodirella longispiculata: Due to lack of personnel, accomplishments on these life histories were limited largely to determining suitable techniques. Also, several attempts to infect lambs with the last-

named parasite by using pronghorn antelope as a source of material were unsuccessful. This failure may be attributed to a difference in strains of this parasite. New Mexico sheep do not harbor N. longispiculata but the incidence is quite high in sheep in Wyoming.

On the Occurrence of Liver Fluke in Arizona Sheep: Five of 49 sheep from 9 different farms or ranches in eastern Arizona were found to be infected with the common liver fluke. The 5 infected sheep were from 2 farms which used small snail-infested ponds as the sole source of drinking water for the sheep.

Anthelmintic Trials against the Fringed Tapeworm: Work continued from last year on the evaluation of bithionol pointed up the high efficacy of this compound. The optimum dose rate was determined to be 220 mg/kg. Twenty-four infected sheep were treated at this rate and at necropsy only 4 harbored tapeworms; at the same time, 19 of 21 controls were infected. Trials with the compounds, Freon 112, Freon 113, and hexachlorophene, showed that they were ineffective against Thysanosoma.

Effect of Freon 112 in Removing Common Liver Flukes from Sheep: This compound was given to 3 fluke-infected sheep at the rate of about 600 mg/kg. At necropsy, only one of these animals harbored flukes, the number being 2. Two untreated sheep examined at this time both harbored flukes, the number being 2 in one case and 23 in the other.

In 1962, at the University Park Field Station, with informal cooperation with the Experiment Station and Regional projects, the following research was reported:

Immunization of Lambs with a Relatively Non-pathogenic Strain of Haemonchus from Pronghorn Antelope: Experiments carried out under laboratory conditions for the second successive year indicated that it may be possible to immunize lambs against sheep strain haemonchosis by inoculating them with a relatively non-pathogenic strain of Haemonchus from pronghorn antelope. Intensity of infection following immunizing inoculations as reflected by the degree of anemia was significantly less in antelope strain lambs than in sheep strain lambs. The responses of the lambs in the two groups to challenge with the sheep strain 63 to 64 days after the initial inoculations did not differ significantly, thus indicating that the antelope strain lambs were practically as resistant to challenge as the lambs which received the homologous strain.

Field Trials on the Immunization of Lambs Against Haemonchus: Field trials were conducted to determine the value of a relatively non-pathogenic strain of Haemonchus for immunizing lambs against Haemonchosis. Lambs receiving immunizing inoculations had consistently higher worm egg production and lower hemoglobin levels than uninoculated controls.

Life History of Thysanosoma: Studies on insects of the order Corrodentia continue to show promise in solving the life history of Thysanosoma but to date no lambs have been infected experimentally with cysticorcoids dissected from these insects.

Effect of Bayer 2353 in Removing Thysanosoma from Sheep: Bayer 2353 at a dose rate of 400 mg/kg failed to remove significant numbers of Thysanosoma from 9 aged ewes and 5 lambs as compared with 8 aged ewes and 5 lambs which served as untreated controls.

Effect of Bayer ME-3625 in Removing Thysanosoma from Aged Ewes: Bayer ME-3625, at a dose rate of 4.4 to 5.6 mg/kg, failed to have a consistent and marked effect in removing fringed tapeworm from 4 aged ewes as compared with 3 untreated controls.

The Occurrence of Liver Flukes in Sheep in New Mexico: The incidence of common liver flukes in 3 farm flocks in New Mexico was found to be 53 percent in 38 ewes examined.

Probable Intermediate Snail Host of Liver Flukes in New Mexico: Circumstantial evidence indicates that the snail Fossoria Modicella is a vector of liver flukes in New Mexico.

Effect of Freon 112 in Removing Common Liver Flukes from Sheep: A controlled test involving 12 aged sheep harboring liver flukes was carried out to ascertain the anthelmintic efficacy of Freon 112 when given at a dose rate of 600 mg/kg. The results confirmed previous observations that the compound is highly effective in removing adult flukes. No immature flukes were present in the host animals.

G. Biology of the Liver Fluke.

In 1961, under cooperative agreement with the Montana Agricultural Experiment Station at Bozeman, a study was conducted on the taxonomy of possible small snail hosts of Fasciola hepatica in Montana. Six species in three families, and four genera were tentatively identified. Several species of another family were collected but were not placed in species, as they probably do not serve as intermediate hosts of Fasciola hepatica. Snails were also collected from 15 locations in Montana, identified as to genera, crushed and examined in the laboratory to determine the trematodes that were present in them. Trematodes were found in those from 4 locations. Numerous methods were tried for the culturing of snails in the laboratory. The best method found for maintaining snails was a modification of that of Taylor and Morley (1948). This consisted of using a sloping grade of clay in the bottom of an 8-inch fingerbowl, covering the lower part of this grade with water and seeding the whole thing with algae. Food, consisting of powdered wheat germ and calcium carbonate, seemed to satisfy the snails. Not much growth and no reproduction was noted on this culture but the snails did survive up to 4 months. With the cooperation of the State Veterinarian and 14 meat inspectors, a survey was made of the distribution of liver flukes in Montana. It was found that liver condemnations totaling 408 were made this year from 189 ranches in Montana from F. hepatica infection, and 11 condemnations of livers were made from 7 ranches due to F. magna infections.

In 1962 this work with Montana was discontinued and a cooperative agreement on bovine coccidiosis was drawn up to replace it with the Montana Agricultural Experiment Station.

H. Effect of Intestinal Roundworms on Metabolism.

In 1961, under a cooperative agreement with the North Dakota Agricultural Experiment Station at Fargo, a study was made on the effect of gastrointestinal nematodes on the wool and sulfur metabolism in lambs. Twenty-five thousand larvae of the Trichostrongylus species were given to each of 8 lambs. Eight lambs were kept as controls. Wool samples from the 16 animals, taken at the initiation and termination of the trial, were analyzed for sulfur content and tensile strength. Plasma protein levels of the animals were determined at 2-week intervals throughout the trial. Ova counts were determined at the terminal stage of the experimentation. The control animals averaged 0.5 ova/gram wet feces. The infected animals averaged 637.5 ova/gram feces.

In 1962, continuing the cooperative research at the North Dakota Station, eight lambs were infected with gastrointestinal nematodes and 8 lambs served as noninfected controls. There were no significant changes in plasma protein levels, white blood cell count, hematocrit and hemoglobin levels. The tensile strength of the wool fibers was adversely affected by nematode infection and the sulfur content of the wool was decreased.

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AREA NO. 13 - PARASITES AND PARASITIC DISEASES OF POULTRY

Problem. Parasites and parasitic diseases probably cost the poultry industry at least $40 million annually, by causing intestinal disturbances, emaciation, retarded growth, reduced egg production, and deaths. Parasites are ubiquitous, many times insidious, and often overlooked until birds are damaged irreparably. Early diagnosis is difficult, and reliable treatments for many devastating parasitoses are not available. Moreover, some management practices intended to avoid spread of parasites and to control them have been found ineffectual, as is shown by the increasing importance of certain parasites in broiler production. The problem is to develop, through a planned, balanced program of basic and applied research, methods for preventing, controlling or eradicating parasitic diseases, thus affording economical production of healthy poultry and sound products in supplies adequate to meet the needs of an expanding population.

USDA PROGRAM

The Department has a continuous long-term program involving parasitologists, biologists, and chemists, engaged in both basic studies and the application of known principles to the solution of the problem of parasites and parasitic diseases of poultry.

The Federal scientific effort devoted to research in this area totals 5.5 professional man-years. This effort is applied as follows:

Bionomics of Intestinal Protozoan Parasites 0.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Immunology of Protozoan Parasitic Diseases 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Control of Coccidiosis 2.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Biology of Nematode Parasites 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 6.2 professional man-years divided among subheadings as follows: Bionomics of Intestinal Protozoan Parasites 0.8; Control of Coccidiosis 3.4; Biology of Nematode Parasites 2.0. Investigations on Bionomics of Intestinal Protozoan Parasites are being conducted on the protozoan causing blackhead in turkeys, the pathogenesis of the disease caused by it in turkeys, and the continuous use of medicaments for the prevention of the disease. The Oregon Station is conducting an investigation on the Control of Coccidiosis to determine the characteristics peculiar

to the different species of coccidia of chickens and the effect of the different species on the host. Several States have studies to evaluate the efficacy of the various coccidiostats. A study on the Biology of Nematode Parasites to determine the nature of the older chicken's resistance to Ascaridia parasitism is under way at the Kansas Station. The Connecticut (Storrs) Station is studying Capillaria columbae parasitism in the chicken - its biology, effects on egg production, and methods of control. The effects of the anthelmintic piperazine citrate on egg yolk quality and egg production is also under study.

Industry and Other Organizations, chemical companies in particular, are engaged in the formulation of compounds and explorations for chemicals that may be used safely as parasiticides. Generally, these companies have their own facilities, including laboratories, poultry houses and other structures containing quarters for poultry. The work of these companies and the results, expenditures, and related matters are ordinarily confidential, since they involve eventually saleable products. Estimated annual expenditures are equivalent to approximately 25 professional man-years. These companies also make grants to State research institutions for investigations of the efficacy and safety of the products.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Bionomics of Intestinal Protozoan Parasites

In 1961 studies at the Beltsville Parasitological Laboratory showed that eggs of the poultry cecal worm, the vector of blackhead, and the blackhead protozoans contained therein were destroyed within one hour when they were immersed in 0.1 percent dilution of Beta-propiolactone (BPL) in water at a temperature of 85 to 90°F. The rate at which these organisms were destroyed by BPL was directly proportionate to the strength applied.

In controlled infections of birds with the nematode, Heterakis, and the protozoan, Histomonas, these nematodes acquired infections of the protozoans only after migrating from the intestinal epithelium into the lumen of the ceca, not during the 9 or so days spent in these tissues immediately following infection. In the female heterakids so infected, only about 1 of every 600 to 3,000 eggs that developed in the bodies of these females contained histomonads, it was calculated. Not all worms developed eggs containing histomonads, however. This calculation was based on findings from quantitative feeding tests with eggs from the Heterakis worms.

In tests this year acute blackhead was acquired by susceptible birds ranged on yards that became naturally contaminated with Heterakis and Histomonas 4 years previously, and which had been protected each summer by growths of vegetation. These tests are part of a long-range ecological study designed to lay a foundation for recommendations for the control of blackhead by rotation of ranges, in the absence of treatment of birds and other procedures which might aid in controlling this disease.

That the control of run-off from dashing rains may be an important management procedure in preventing the dissemination of blackhead from contaminated yards

to clean ones was shown by observations made during a period of several years. Eggs of the vector Heterakis survived and, after being washed onto clean plots, transmitted blackhead to susceptible birds ranged thereon. Plots with vegetation onto which the washings from contaminated plots flowed proved the most dangerous, because the vegetation impeded the flow of water, providing opportunity for the eggs to settle onto the soil where they became available to birds.

Observations this year indicated that oocysts of cecal coccidia of chickens and turkeys are more resistant to deleterious climatic factors than the oocysts of intestinal coccidia. In general, the survival of the former on experimental plots was approximately twice as long as the latter under similar conditions. On experimental plots, eggs of Heterakis containing Histomonas were found to have survived 78 and 73 weeks on shaded and unshaded soil plots, respectively. In the crop of chickens oocysts of an intestinal coccidium appeared to undergo no readily detectable changes. In the gizzard the oocyst shells ruptured, releasing the sporocysts (sac-like bodies containing the sporozoites which are invasive bodies). The latter did not emerge from the sporocysts until after these bodies had reached the duodenum, the site of infection. In the case of a cecal coccidium of turkeys, release of sprozoites was delayed until after the sporocysts were transported to the region of the yolk stalk or posterior to it.

A comparison was made of the in vitro excystation of oocysts of E. acervulina, E. gallopavonis, E. meleagrimitis, and E. tenella. The oocysts of E. acervulina and E. meleagrimitis, parasites of the duodenum, excysted much quicker than did those of E. tenella and E. gallopavonis which are parasites of the cecum. These in vitro studies parallel findings from in vivo studies in that sporozoites of E. tenella and E. gallopavonis were never found in the duodenum of test birds, whereas those of E. acervulina and E. meleagrimitis were always recovered from that location at necropsy. These findings illustrate the perfect adaptation of these four coccidia to their hosts.

In 1962 studies at the Beltsville Parasitological Laboratory showed that in chickens one through four days of age the numbers of coccidial oocysts that hatched (excysted), the number and severity of lesions in the small intestine, the effect on the bird, and the number of oocysts produced per oocyst fed were least in the youngest birds and greatest in the oldest. It was also observed that a more severe coccidial infection resulted when oocysts were inoculated directly into the crop than when they were introduced into the small intestine. In chickens and turkeys it was shown that the oocysts begin hatching in the gizzard and is completed in the small intestine. The species that parasitize the small intestine survived the action of digestive enzymes in less time than those that parasitize the blind gut and surrounding areas. Two species each of chicken and turkey coccidia were used. In turkeys, blackhead begins usually from 4 to 18 days after the vector eggs are swallowed. The pathogenic form of the blackhead agent was proved to be more easily adapted to the vector, the cecal worm, than the nonpathogenic form.

The nonpathogenic variant of the blackhead organisms was determined to be a new species and given the name Histomonas wenrichi.

Studies on the in vitro excystation of sporozoites of poultry coccidia from mechanically released sporocysts showed that excystation is probably due to enhanced (by the action of bile salts) enzymatic activity of trysin, chymotrypsin, and possibly lipase. The excystation rate induced by alpha- and beta-chymotrypsin was comparable to that obtained with an impure pancreatic preparation, trypsin 1-300, and much greater than that obtained with a highly purified preparation trypsin 2x. Significantly, carbozypeptidase A or carboxypeptidase B used alone, or in combination with a bile salt, failed to induce excystation. This selective enzymatic action suggests that the Stieda body (sporocystic plug), upon which the enzymes act, is composed, in part, of long-chained peptid linkages.

In connection with attempts to cultivate in vitro the poultry coccidium, Eimeria acervulina, in the duodenal cells of the host, a method was developed whereby chicks could be hatched and raised to 6 days of age with a much reduced intestinal flora. Cultures, prepared from duodenal tissue of such chicks, grown in a medium containing standard amounts of antibiotics (100 units of penicillin G and 300 micrograms of dihydrostreptomycin sulfate) were bacteria-free.

Gizzards of 1-day-old chicks which have less well developed musculature and smoother linings than gizzards of 2- and 3-day-old chicks, proved less susceptible to coccidial infections than older birds. A very low percentage of sporocysts were released from oocysts in gizzards of 1-day-old chicks and a low percentage of sporozoites escaped from liberated sporocysts in the small intestine. Two-and 3-day-old chicks were progressively more effective in excysting oocysts. A motion picture on the excystation (hatching) and locomotion of sporozoites of poultry coccidia was prepared in collaboration with scientists of the UCLA.

B. Immunology of Protozoan Parasites.

In 1961 at the Beltsville Parasitological Laboratory, in work to control blackhead by immunization against its vector, the poultry cecal worm, the incidence of blackhead was 37 percent lower in a group of poults so immunized and run on a plot infectious for cecal worms and blackhead, and 42 percent fewer birds died from blackhead than was the case in a comparable, non-immunized control group. The number of cecal worms recovered from the immunized birds at necropsy was 46 percent less than from the controls. "Immunization" against these worms was accomplished by the administration to each bird of 100 embryonated eggs known to be free of blackhead parasites.

Experimental work with the turkey coccidium Eimeria gallopavonis, revealed that this parasite is capable of causing sickness, loss of weight, and death of affected birds as old as 7 weeks. That portion of the intestinal tract surrounding the cecal juncture was generally affected most severely. Birds that survived an initial infection were found to possess a high degree of

resistance to subsequent massive infections of this parasite. After the oocysts were swallowed by susceptible birds approximately 144 to 150 hours were required for the parasites to excyst, complete their cycle of development, and for new oocysts to appear in the droppings.

In work to develop a procedure that could be used in immunizing chickens against several species of coccidia at one time, a single dose of coccidial oocysts comprised of three or four of the more pathogenic species, did not confer a satisfactory degree of resistance to any one or to all of the species involved. This is shown by the fact that all these birds "challenged" by means of an inoculum containing three species (E. tenella, E. necatrix, and E. acervulina) or four species (E. tenella, E. nacatrix, E. maxima, and E. acervulina) exhibited pronounced clinical symptoms of coccidiosis severe enough to result in death of a sizeable number of the birds involved.

In 1962 at the Beltsville Parasitological Laboratory, studies showed that a single infection of turkeys with the coccidium Eimeria gallopavonis can be harmful to birds 3 to 12 weeks old. However, turkeys that survived an initial infection of the coccidium were immune to a second (challenge) infection of this parasite, administered 29 days after the first. None of the immunized birds became sick, none died, and the digestive tract was undamaged. Of the control, nonimmunized birds, 20 to 100 percent died, with severe infections.

C. Control of Coccidiosis.

In 1961 at the Beltsville Parasitological Laboratory, Beltsville, Maryland, in studies of the reaction of the cecal coccidium Eimeria tenella to drugs recommended for treatment, strains of this parasite having varying degrees of resistance to nicarbazin, Unistat, Trithiadol, nitrofurazone, and arsenosobenzene were produced experimentally. An experimentally developed strain resistant to nitrofurazone was tested for cross-resistance to Unistat, nicarbazin, arsenosobenzene, glycarbylamide, Trithiadol and Zoalene, but no evidence of cross-resistance was observed. Studies to develop a strain of E. tenella resistant to amprolium were unsuccessful.

Preliminary work with terephthalic acid as a "potentiator" of aureomycin administered in feed for the control of cecal coccidiosis of chickens yielded promising results. A combination of terephthalic acid (0.5 percent) and aureomycin (0.0055 percent) in feed afforded 3-week-old chickens complete protection against mortality from experimentally induced coccidiosis and enabled the infected birds to grow at a rate comparable to that of uninfected, unmedicated controls under experimental conditions. Neither compound by itself administered in feed afforded demonstrable protection to birds under experimental conditions. The medicated mash was given 24 hours before the birds were infected, and continued for 14 days.

In 1962 at the Beltsville Parasitological Laboratory, strains of Eimeria tenella, serially passed 21 times through groups of chickens receiving nicarbazine, Unistat, Trithiadol, or arsenosobenzine in the feed, developed varying degrees of tolerance to the respective coccidiostats. The decreased sensitivity

was reflected by increased severity of cecal lesions, increased oocyst production, and general unthriftiness of the experimental birds.

Chlortetracycline in the feed at a level of 0.0055 percent (50 grams per ton), when potentiated by 0.5 percent terephthalic acid, gave complete control of cecal coccidiosis in some birds but not in others.

An analysis of the sporulation rates of E. tenella oocysts in droppings of chickens fed various coccidiostats indicated that the chemicals fed did not adversely affect sporulation. When the coccidiostats were mixed with oocyst-containing droppings for laboratory culturing, however, sporulation was inhibited in cultures containing nicarbazin, Zoalene, amprolium, and Unistat.

A strain of E. tenella that was serially passed through chickens fed sub-optimal levels of amprolium developed a tolerance for this low level of the chemical. However, after 9 such passages the strain was still fully susceptible to the usual field level of amprolium.

D. Biology of Nematode Parasites.

In 1961 at the Beltsville Parasitological Laboratory, the discovery of many immature larvae of Ascaridia columbae in the livers of pigeons following heavy experimental infections with this nematode, as reported last year, gave rise to the belief that this parasite may have a migratory phase in its life cycle similar to that of ascarids of non-avian hosts. Tests conducted this year demonstrated that A. columbae does not have a migratory phase outside the digestive tract but completes its development within the small intestine. Larvae encountered in the liver of birds to which large numbers of embryonated eggs of A. columbae were administered were found to become encapsulated and did not leave that organ to complete their development. The additional finding of immature stages of the parasite in the mucosa of the jejunum and ileum indicated that the true migratory phase was limited to the intestinal lining. It was determined that 30 to 35 days were required for A. columbae to complete its life cycle in the pigeon.

A severe outbreak of gapeworm infection causing death losses in pheasants was studied. Feeding turkey poults the earthworm Helodrilus foetida and H. caliginosus from the soil of the pen in which the pheasants were confined established the fact that these worms were an important source of infection. Eight of 12 turkey poults failed to survive the feeding of 10 of the gapeworm-infected earthworms and at necropsy harbored about 50 pairs of gapeworms per bird. The average weight of the 4 surviving infected birds was 171.9 grams less than that of the uninfected controls at the termination of the experiment at the end of 25 days. In cooperation with the Nematology Section of the Plant Industry Station, an experiment was carried out to determine the effectiveness of soil fumigants for the control of gapeworm infection by destroying infected earthworms in the soil. Selected plots (10 feet square), known to contain infected earthworms, were treated with methyl bromide or with DD, a mixture of Dichloropropene and Dichloropropane. One pound of methyl bromide per 100 square feet of soil surface killed about 98 percent of the earthworms in the top 8 inches of soil when it was applied under a vapor-proof cover. When

DD was applied at the rate of 30 and 50 gallons per acre, respectively, by means of an applicator, it was relatively ineffective.

Cockroaches are known to serve as intermediate hosts of certain species of helminth parasites of birds. An attempt was made to infect three species of roaches, Periplaneta americana, Supella supellectilum, and Blatella germanica, with eggs of the gapeworm, Syngamus trachea, mixed in feed. Turkey poults became infected with this parasite when fed P. americana and S. supellectilum 2 days after exposure. Turkeys fed similarly exposed B. germanica did not become infected.

In order to expedite experimental work with poultry helminths, methods of culturing embryonated eggs and of hatching them in vitro were investigated. Dilute aqueous solutions of formalin (0.2 to 6.0 percent) were used in attempts to increase the infective larvae developing in the unhatched eggs of Capillaria obsignata. Only a small percentage of these eggs were infective to chickens when cultured in this way. The largest percentage hatched and continued their development when cultured in 0.5 percent formalin.

In a study of the hatching mechanism of the eggs of Heterakis gallinae, a number of female worms containing embryonated eggs were fed in No. 2 capsules to five 4-week-old chicks. The birds were killed 1, 2, 2-1/2, 3, and 4 hours after ingesting the capsules. At the end of 1 hour, unbroken capsules containing intact worms were recovered from the crop; in 2 to 2-1/2 hours unhatched eggs and fragments of worms were recovered from the gizzard; in 3 hours larvae were recovered from the ileum, but no unhatched eggs or larvae were seen elsewhere in the intestine; and by the fourth hour, larvae were found near to the entrance to the ceca. These observations indicated that the gizzard functions to break up the capsules and the worms thus freeing the eggs for action by the digestive juices of the intestine.

Young chicks placed on litter contaminated with the eggs of the large intestinal roundworm of chickens, Ascaridia galli, and the threadworm of chickens, turkeys, and pigeons, Capillaria obsignata, and exposed to such litter for two periods of 5 and 4 days each, with an intervening period of 10 days, weighed on an average of 92.1 grams less than similar control birds kept on clean litter. Although the infected birds did not become heavily infected, (1 to 11 A. galli and 1 to 4 C. obsignata) the acquisition of parasites and the exposure to used litter points up the fact that chickens require sanitary conditions for their best development.

In 1962, in studies at the Beltsville Parasitological Laboratory, the life cycle of Ascaridia columbae, the large intestinal roundworm of the pigeon, was completed experimentally for the first time under controlled conditions. The embryos within the eggs developed to the infective stage in approximately two weeks at room temperature. In the pigeon, the larvae attained the third stage of development between the third and sixth days, the fourth stage between the 11th and 14th days, the fifth stage at about the 17th day, and eggs appeared in the feces 37 to 42 days after experimental infection. This work also demonstrated that the parasitic phase of the life cycle was completed entirely within

the alimentary tract. Although larvae were observed in the liver, portal vein, bile duct and lungs of experimentally infected pigeons, no evidence was obtained to support the hypothesis advanced earlier in the investigation, that a migratory phase outside the digestive tract might be necessary for the completion of the life cycle. Histological examination of the lesions produced in the liver by migrating larvae of Ascaridia columbae disclosed that their cellular composition changed with the age of the infection.

Methyl bromide was demonstrated to be an effective soil fumigant for the destruction of the earthworm intermediate hosts of the poultry gapeworm, Syngamus trachea, for the control of this parasite in pheasants.

Thiabendazole, a new drug having the chemical formula 2-(4-Thiazolyl)-benzimidazole, when mixed in mash in the proportion of 0.3 percent of a 10 percent premix by weight and fed continuously for 12 days was not effective in removing the large roundworm, Ascaridia columbae, and the intestinal redworm, Ornithostrongylus quadridadiatus, from pigeons. However, it sharply reduced worm egg production of both species, but had little or no effect on the production of oocysts by coccidia.

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AREA NO. 14 - TREATMENT FOR REMOVAL OR CONTROL OF PARASITES OF DOMESTIC ANIMALS

Problem. Parasites of food animals are responsible for losses to livestock producers approximating a billion dollars annually. This estimate, moreover, is conservative since it does not take into account costs of treatment and other control measures. Chemical antiparasitic agents are the most powerful weapons presently available against parasites and the diseases they cause, yet, specific treatments generally have a comparatively short period of usefulness. Many of the currently preferred treatments were unknown a decade or so ago and, in all probability few, if any of those in use today will be primary choices a decade or so hence. Moreover, the growing concern with respect to residues in edible tissues and organs of treated animals and birds necessitates development of control measures other than treatment. The problem is to develop, through a planned, balanced program of basic and applied research control methods that minimize reliance on extrinsic chemicals. These include investigations of immunological procedures, management practices which minimize exposure of animals to parasitic infections, and natural control agents such as parasites, pathogenic microorganisms, and predators of economically important livestock pests.

USDA PROGRAM

The Department has a continuing long-term program involving veterinarians, parasitologists, pharmacologists, and biochemists engaged in both basic studies and the application of known principles in developing treatments for removal or control of parasites of domestic animals. Research is being conducted on this problem at the following designated locations.

The Federal scientific effort devoted to research in this area totals 13.0 professional man-years. This effort is applied as follows:

Chemical Control of Parasitic Diseases 1.5 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

New and Improved Anthelmintics 3.0 at the Beltsville Parasitological Laboratory, Beltsville, Maryland.

Hazards of Residues from Treatment for Parasites 3.5 at the Regional Animal Disease Research Laboratory, Auburn, Alabama.

Parasitic and Related Skin Diseases 1.5 at the Albuquerque, New Mexico, field station.

Pathobiology of Parasitic Infections 1.0 at the Albuquerque, New Mexico, field station.

Methods for Control and Eradication of Ticks 1.0 at the Albuquerque, New Mexico, field station.

Control and Eradication of Scabies 1.5 at the Albuquerque, New Mexico field station.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

State Experiment Stations in 1961 reported a total of 5.7 professional man-years under the following subheading:

Treatment for Removal or Control of Parasites of Domestic Animals 5.7. Regional research (S-21, Gastrointestinal Parasites of Ruminants and W-35, Nematode Parasites of Ruminants) is providing a scientific basis for chemical control of ruminant parasites. These studies seek to establish efficacy of new compounds against various important parasite species. Methods of administering the compounds and dosages required for most effective parasite control are evaluated along with considerations of toxic effects of the compounds on host animals. Procedures for simplifying the administration of anthelmintics are being developed and their use coordinated with effective management practices. Studies are developing fundamental information on how anthelmintics act upon the metabolic processes of parasites to impair their reproductive processes or result in their death and elimination from infected animals. The problem of drug resistance which has been found to occur in roundworms subjected to prolonged periods of exposure with small amounts of anthelmintics is also being studied. New compounds are being evaluated for use in controlling the spread of trichomoniasis from bulls. Drug dosages, methods of application and frequency of administration are under study. Serological procedures are being studied for possible application in diagnosing the disease.

Industry and Other Organizations, especially chemical companies, are engaged in research programs of varying magnitude designed to discover and develop antiparasitic formulations. These companies have laboratory facilities of their own for preliminary investigations and, to a greater or lesser degree, animal facilities (barns, pens, pastures, etc.) for limited definitive trials. Some of their experimental testing is carried out under contract with State experiment stations and universities; large-scale field trials are occasionally conducted under cooperative agreements with livestock producers. Much of this work is carried out on a confidential basis, the results being unknown to other researchers, veterinarians, and livestock interests. Estimated annual expenditures are equivalent to approximately 110 professional man-years in this broad area which includes livestock in general as well as poultry.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Chemical Control of Parasitic Diseases.

In 1961 at the Beltsville Parasitological Laboratory, strains of Eimeria tenella that were serially passed 16 times through groups of chickens receiving nicarbazin, Unistat, Trithiadol, or arsenosobenzene showed varying degrees of resistance to the respective drugs as evidenced by increased severity of cecal lesions, increased oocyst production, and decreased weight gains. Furthermore, deaths occurred occasionally in the groups that were given Trithiadol or Unistat. Drug tolerance was especially pronounced in a strain that was serially passed through 12 groups of nitrofurazone-medicated birds. There was no evidence of cross-resistance, however, when this strain was subsequently tested with Unistat, nicarbazin, arsenosobenzene, glycarbylamide, Trithiadol, and zoalene. These findings were of particular interest because, as previously reported (Cf. 1959 and 1960 Reports), glycarbylamide- and zoalene-resistant strains of E. tenella were also resistant to nitrofurazone.

Studies were continued in sheep with pure infections of Haemonchus contortus (Cf. 1960 Report) to further investigate strain variation in the response of this parasite to phenothiazine. Tests were made with two commercial phenothiazine preparations (purified and N.F. grade products each with an average particle size of about 7 microns) to obtain comparative data on the therapeutic action as well as on the effect of these preparations on egg production and larval development.

Available data at this time suggest that purified phenothiazine may be somewhat more effective than N.F. grades of comparable particle size against resistant strains of Haemonchus. Very little difference in efficacy was noted between these preparations in trials with non-resistant strains of the parasite, each being 99 to 100 percent effective at the 0.25 gram dose level per pound body weight. The purified product, moreover, was completely effective against non-resistant adult worms when given at the rate of 0.125 gram per pound of body weight, whereas the N.F. product at this dose rate was 93 to 98 percent effective. In trials with the Kentucky resistant strain, 0.25-gram dosages of purified and N.F. grade products were 95 and 79 percent effective, respectively; and against the Maryland resistant strain, the corresponding percentage efficacies were 84 and 13 percent, respectively.

In 1962 at the Beltsville Parasitological Laboratory, a strain of Eimeria tenella that had been passed five times through groups of chickens fed 0.00125% amprolium (one tenth the manufacturer's then recommended level) was fully susceptible to the field level of the chemical. This strain has now been passed nine times; from the sixth passage on it has been propagated in birds fed mash containing 0.0025% amprolium. Detectable resistance has developed to this level of the coccidiostat. However, when oocysts recovered from the ninth passage were fed to birds receiving 0.0125% amprolium in the mash, oocyst production was practically nil, gross pathological changes in the ceca were non-existent and there was no detrimental effect on weight gain.

Observations and analysis of the available data suggest that purified phenothiazine may be somewhat more effective than N.F. grades of comparable and larger particle size against resistant strains of Haemonchus contortus. Very little difference in efficacy was noted between preparations of comparable particle size in removing non-resistant strains of the parasites. At dose rates of 0.25 gram per pound body weight, 98 to 100 percent of the adult worms were removed in trials against normally responsive Kentucky and Maryland strains. The purified product, moreover, was 96 to 99 percent effective against both strains at the rate of 0.125 gram per pound body weight, whereas the efficacy of the comparable N.F. product at this dose rate was 88 and 93 percent, respectively. In trials with the resistant Kentucky strain, 0.25-gram dosages of purified and N.F. grade products were 95 and 79 percent effective, respectively; and against the resistant Maryland strain, the corresponding percentage efficacies were 85 and 84 percent, respectively. Similar analyses of the 0.125 gm-level show the purified and N.F. products were 78 and 55 percent effective, respectively, against the resistant Kentucky strain, and 74 and 14 percent effective, respectively, against the resistant Maryland strain. In additional trials with the resistant Maryland strain the purified and N.F. products were 99 and 57 percent effective, respectively, at the 0.5-gram dosage level. The purified chemical at this level was 99 percent effective with the resistant Kentucky strain. Resistance to the N.F. product by the tolerant Maryland strain was further demonstrated at dosage levels of 1.0 and 1.5 gram per pound body weight. Reductions in egg production and larval development followed patterns corresponding to those for the removal of adult worms.

B. New and Improved Anthelmintics.

In 1961 at the Beltsville Parasitological Laboratory, studies showed that Ruelene (Dow M-1261), an organic phosphate, given orally to 2 steers at a dose rate of 60 mg/kg, was highly effective against H. placei, C. punctata, O. radiatum, and T. axei. An emulsifiable formulation (Dow M-1609), poured along the dorsal mid-line of 6 steers at a dose rate of 75 mg/kg, showed some anthelmintic activity; however, its activity was extremely erratic from species to species, and from animal to animal.

Thiabendazole (Merck MK-360) was found to be extremely effective as an anthelmintic in sheep against H. contortus, T. columbriformis, S. papillosus, and Oesophagostomum spp.

An experiment at Poplarville, Mississippi, during 1960 was carried out with 44 head of cattle to test the anthelmintic properties of Ruelene formulation M-1609. The drug was administered by pouring it along the mid-line of the backs of the cattle. The treatment appeared to have some activity against Haemonchus placei, Cooperia spp., and Oesophagostomum radiatum.

In an experiment involving 180 lambs, the anthelmintic activity of phenothiazine, copper sulfate-nicotine sulfate, Ruelene, Co-Ral, and Trivermol was tested. Co-Ral, at a dosage of 7.5 mg/kg of body weight was the most effective in reducing worm numbers, and Ruelene was next best. Trivermol was totally ineffective at the recommended dosage.

Control of over 95% of cattle grubs was obtained treating animals on the 1st of August with an 0.5% Ruelene spray and a Ruelene "Pour-On" formulation at 50 mg/kg, and 75 mg/kg. The animals treated with the "Pour-On" formulation gained more weight than those that were sprayed or left untreated. The peak emergence of grubs in Georgia seems to be around the latter part of January.

An experiment was conducted to compare the efficiency of Ruelene formulation M-1782, with Ruelene formulation M-1560, in the control of gastrointestinal nematodes of sheep. The number of eggs in feces obtained from the sheep was greatly reduced by both formulations, as compared to a control group. The number of eggs being passed by the sheep in the 2 treated groups was much greater than the number from the control group 63 days post-treatment.

Four hundred and eighty lambs, and 520 ewes were treated with 3/4 oz. and 1 oz., respectively, of Ruelene formulation M-2097 (Dow Chemical Company) in a toxicity trial in sheep. One lamb was found dead 24 hours after treatment, but the rest appeared normal until sold 20 days post-treatment.

Two dose levels of Ruelene "Pour-On" formulation M-1609 significantly reduced the number of grubs emerging in the backs of yearlings. The number of nematode eggs in the feces obtained from the treated animals was also significantly reduced, as compared to the control group. However, the low egg counts observed from all animals inject a degree of uncertainty as to the reliability of the results.

Additional critical data were obtained on the anthelmintic action of dithiazanine iodide in dogs and cats. In the aggregate, single dosages of 12.5 mg/kg body weight removed 46% of 52 ascarids from 2 dogs, and 39% of 1,128 hookworms, and 5% of 599 whipworms from 5 dogs. Efficiency against hookworms and whipworms increased markedly when this dosage was given daily for 5 days. In these trials, the chemical removed 60% of 1,581 hookworms, and 80% of 1,141 whipworms from 5 dogs. None of the animals harbored large intestinal roundworms. When the dose rate was increased to 25 mg/kg, and given twice on one day, the chemicals removed 31% of 2,279 hookworms, and 67% of 851 whipworms from 2 dogs. With this dosage also efficiency was substantially increased when treatment was given on 5 successive days. In these trials, the drug removed 72% of 1,678 hookworms, and 85% of 2,539 whipworms from 5 dogs, and all of 28 ascarids from 2 dogs. The dosage of 25 mg/kg, given twice on one day, removed 88% of 42 ascarids from 5 cats. By and large, the several regimens were reasonably well tolerated, although treatment was generally followed by the passage of stained, mucoid feces.

In limited trials with dogs, a new systemic anthelmintic, methyridine, removed 75% of 200 hookworms from 4 dogs, and all of 220 whipworms from 3 dogs. The chemical was given by subcutaneous injection at the rate of 200 mg/kg of body weight. Emesis and signs of transient intestinal discomfort were noted in all of the test animals.

A solution of the organophosphate compound Bayer 13/59 (Dipterex, Dylox) removed 65% of 833 hookworms and all of 259 whipworms when given by intramuscular injection to 2 dogs at the rate of 100 mg/kg of body weight. A similar regimen was completely effective against 12 ascarids in 1 cat, but failed to remove the 2 Dipylidium from this animal. One of the dogs vomited shortly after treatment and all animals exhibited a temporary muscular incoordination.

In 1962 at the Beltsville Parasitological Laboratory, thiabendazole, a new anthelmintic for livestock, appeared to be unusually effective against most of the common gastrointestinal parasites of sheep. Thiabendazole, 50 mg/kg body weight, in gelatin capsule, methyridine, 200 mg/kg body weight, by subcutaneous injection; and 2-3 micron purified phenothiazine, 550 mg/kg body weight, as a drench, were studied in comparative trials using flock ewes with moderate to light mixed parasite infections. Preliminary findings indicate marked action against Ostertagia, Trichostrongylus, Cooperia, Nematodirus, and Oesophagostomum. In one series of trials with flock ewes held on concrete several weeks before treatment, the chemical thiabendazole was adjudged to be about 94 percent effective in removing gastrointestinal parasites compared to 83 percent efficacy, and 45 percent efficacy of purified phenothiazine. Mature Haemonchus were recovered from all groups of animals including the thiabendazole-treated ewes. In trials with ewes taken off pasture and treated, thiabendazole was adjudged 99 percent effective, methyridine was adjudged 12 percent effective, and purified phenothiazine was 74 percent effective. Immature Haemonchus were recovered from all groups of principals and the controls; mature Haemonchus were recovered from the methyridine, phenothiazine, and control groups, but not from the thiabendazole-treated group. No toxicity was observed.

Low-level daily doses of 250 mg. thiabendazole given for 20 days caused a marked reduction in ova per gram feces and numbers of larvae recovered from baermannized cultures of feces. When administration of the drug was discontinued, both egg and larval counts increased but not to pre-treatment levels.

Thiabendazole is highly effective against H. placei, T. axei, T. colubriformis, and Ostertagia ostertagi. Its activity is extremely variable against Cooperia spp. It is also effective against the fourth-stage larvae of T. axei, T. colubriformis, C. punctata, and C. pectinata but not against the third-stage of O. radiatum.

Famophos (American Cyanamid Co.), an organophosphate, was found to be a highly effective anthelmintic in cattle against H. placei, T. axei, Cooperia spp., Oes. radiatum, and N. helvetianus. In sheep it was equally effective against H. contortus, T. axei, T. colubriformis, and Oes. venulosum.

An experiment was conducted at Natchez, Mississippi, during the summer of 1961 with 160 lambs to test the following drugs for anthelmintic effectiveness at the dose rates indicated: Ruelene topical application at 75 mg/kg; Co-Ral drench, 5 mg/kg, Co-Ral plus Neguvon drench, 4.5 mg. of Co-Ral and

45.5 mg. of Neguvon per kg. of body weight; Co-Ral topical application at approximately 75 mg/kg; phenothiazine, 25 g. per lamb; Famophos drench, 100 mg/kg; Co-Ral plus phenothiazine drench, 60 mg. of Co-Ral and 25 g. of phenothiazine per lamb; and Promintic, 1 cc. of a 90% v/v aqueous solution per 10 lbs. of body weight. Promintic showed the greatest anthelmintic effectiveness and Co-Ral drench at 5 mg/kg was the next most effective. The topical treatments were the least effective of all treatments, and the other treatments were intermediate in their action against the nematode parasites of the lambs.

Famophos at a dose rate of 50 mg/kg was very effective in cattle against Trichostrongylus axei, and somewhat less effective at 25 mg/kg in a small test in Mississippi. The drug was also effective in grub control, especially at the higher dose rate. Weight gains of the treated animals were as high as untreated controls.

A test conducted to determine the anthelmintic properties of Ronnel (ET-57) in two commercial preparations -- "Rid EZY" Salt at a dose rate of 6.6 mg/kg per day for 30 days, and Ronnel Feed-Mix at a dose rate of 15 mg. per kg. per day for 7 days -- showed no anthelmintic activity as used in this test. Specimens of Trichostrongylus longispicularis were recovered from steers native to northern Mississippi.

The anthelmintic properties of two Ruelene formulations (pour-on and feed additive) were compared in cattle. Both formulations were about 60% effective, as determined by comparing the reduction in the number of worms in treated versus control animals. Pour-on appeared to be more effective for the stomach worms, but the feed additive formulation was more efficacious against the intestinal worms.

C. Hazards of Residues from Treatment for Parasites.

In 1961 at the Auburn, Alabama laboratory, studies showed that lambs weaned when 45 lbs. of weight (early-weaned) had a slightly lower number of internal parasites than lambs which remained with their ewes until the slaughter weight of 90 pounds (late weaned). The difference was significant from the first three slaughters, but it was reversed during the last slaughter. This was probably due to the fact that the lambs remaining on Lot 2 could not adequately graze that pasture, which eventually lost much of its nutritional value.

Tests showed that animals on dry lot harbored a significantly higher number of worms than those on pasture plus various supplements. This was probably due to poor management, as short sward was sparsely distributed throughout the area during most of the test and it was readily consumed by the lambs, thus transmitting the parasites. Contrary to last year's findings, the animals on the pelleted ration had fewer parasites than those on the same diet, but in a mash form, and those on mash supplement had more worms than those on pasture alone.

Experiments have been carried out to determine the effect of feed formulations, in mash and pelleted forms, on worm burdens in rabbits infected with 10,560 to 11,020 T. axei and/or T. colubriformis larvae. Worm counts after 20 days infection have not shown that a marked difference in worm burden exists between animals maintained on mash and animals on pelleted ration.

In 1962 at the Auburn Laboratory, studies showed that contrary to the results obtained during two previous grazing seasons, lambs weaned when 45 lbs. of weight (early-weaned) had a higher average number of internal parasites than lambs which remained with their ewes until slaughtered at about 85 lbs. (late-weaned). The low numbers of parasites encountered and the contradictory results may be explained by the facts that all animals were on pasture for a shorter period (33 days) than during previous years. Besides, the abnormally long winter, followed by summertime conditions without the spring-like transition, produced a sudden abundance of forage.

As a result of an unusually dry fall and long winter, followed by a cold spring restricting the length of the grazing season, parasitism was very low in sheep used in four different nutritional and management programs, consequently, there were no marked differences in numbers of gastrointestinal nematodes.

A non-commercial formulation of mash and pelleted ration had no effect on worm burden in experimentally infected lambs and rabbits. Rabbits maintained on a commercial mash and pelleted ration showed a marked difference in worm burden when experimentally infected with T. axei.

Groups of steers grazed on plots of oats and ryegrass each of which had received different summer treatments - fallowed and a crop of Sudan grass for silage - showed no significant differences between summer treatments and pasture types and pasture loads. The steers on oats harbored fewer Trichostrongylus axei than those on ryegrass, both fallowed and Sudan grass summer crop.

D. Parasitic and Related Skin Diseases.

In 1961, at the Albuquerque, New Mexico laboratory, CO-RAL wettable powder sprays were applied to 3 herds, totalling 215 head of cattle. The spray was applied with a Spray-Dip machine to 2 herds, and with a power sprayer to the third herd, delivering the spray at about 400 psi. Ninety-eight percent control of the grubs was obtained with the Spray-Dip machine and 93 percent with the power sprayer. Ruelene emulsifiable sprays, having a concentration of active ingredient of 0.375 percent, was applied to 2 herds composed of 298 head. No control was obtained with the power sprayer, but 84 percent control was obtained with the Spray-Dip machine. No satisfactory explanation is offered for the one complete failure to control cattle grubs with Ruelene at 0.375 percent. Preliminary tests with Famophis, an injectible organic phosphate compound, for the control of larvae of the sheep nosebot, Oestrus ovis, showed very promising results. It was determined to be 100 percent effective biologically against first instars, and 95 percent effective

against all three larval stages. Furthermore, Famophos was found to possess a wide margin of safety.

One hundred seventy-two sheep were examined for incidence of infestation with larvae of the sheep nosebot, Oestrus ovis. Only 17 were free of parasites. The infestations were found to be made up of first, second, and third instars, in percentages of 79, 13, and 7, respectively. Single annual treatment of a flock of isolated range sheep with injectible dimethoate for the control of Oestrus ovis started in 1958, show interesting results. Before treatment in the fall of 1958, the flock was determined to be 100 percent infested, with an average of about 59 larvae per animal. In 1959, the average number of larvae was reduced to an average of 26 per head. In 1960 a slight increase in the average number of larvae per head was recorded, and was attributed to the fact that the entire flock was not treated. In fact, only about 28 percent of the animals were treated.

Dimethoate for the control of Oestrus ovis larvae infesting sheep was found to be less effective when administered subcutaneously as compared with the intramuscular route. Although the dosage administered subcutaneously was about 55 percent greater, the reduction in average number of larvae and the reduction in the percent of infested animals was less than when a lower dose was administered intramuscularly. In a limited but controlled test, it was found that dosages of dimethoate, ranging from 16 - 18.9 mg/kg were 100 percent effective against first instars of Oestrus ovis. Field tests with injectible dimethoate show that sheep under New Mexico range conditions cannot tolerate dosages of 25 mg/kg. They can be treated safely with 20 mg/kg. Nearly 100 percent effectiveness is attained against first instars.

Low level feeding of ronnel, an organic phosphate insecticide developed for the control of cattle grubs and other livestock ectoparasites, was found ineffective against psoroptic scab mites of sheep. Dosage levels of 5 mg/kg a day for 30 days, and 10 mg/kg for 20 days, had little or no effect on the mites.

In 1962 at the Albuquerque, New Mexico, laboratory, tests to control cattle grubs, Hypoderma bovis and H. lineatum, showed that very high levels of control could be obtained with 0.25 percent CO-RAL applied with the Spray-Dip machine. An interesting feature of the treatment was the use of the grub rake, which apparently enhanced the effectiveness of CO-RAL. Tests in New Mexico with Bayer 29493 showed that at a concentration of 0.15 percent, it was as effective as CO-RAL at 0.5 percent, when applied as a spray.

Dimethoate for the treatment of sheep infested with Oestrus ovis was toxic to the sheep at 13 - 25 mg/kg, despite all measures taken to prevent intoxication. Therefore, this highly effective compound cannot be recommended for treatment of this parasite under range conditions.

Tests with Famophos for the control of Oestrus ovis show considerable promise as a treatment for sheep infested with this parasite. No toxicity was encountered at dosages of from 40 - 70 mg/kg. More than 95 percent effectiveness against first instars was obtained with doses of approximately 50 mg/kg. More precise data on the effectiveness of Famophos against second and third instars will likely be obtained if evaluation of the results is made a week after treatment rather than 72 hours as has been done with dimethoate.

Topical application of CO-RAL, Bayer 29493, and Famophos for the control of O. ovis indicates that 97 percent control can be obtained with Bayer 29493. The other two compounds were ineffective. The need for additional tests with this compound and the method of application is indicated.

Biological tests with O. ovis larvae show that third instars can be kept alive for as long as 62 days, providing bacterial contamination is kept at a minimum, and the temperature at about 10°C. Second instars were kept alive 49 days under similar conditions. Larvae food requirements, optimum conditions of temperature and RH have as yet not been determined.

E. Pathobiology of Parasitic Infections.

In 1961 at the Albuquerque, New Mexico, laboratory, low level administration of ronnel, an organic phosphate compound recommended for the control of cattle grubs and other livestock ectoparasites, was found to be ineffective against the blood sucking louse of cattle, Hematopinus eurysternus. Dosage levels of 5 mg/kg/day for 27 days and 10 mg/kg/day for 22 days, were found to be ineffective in controlling lice on louse-carrier animals.

Aqueous sprays of CO-RAL having a concentration of 0.1 percent were found to eradicate an infestation of Damalinia bovis in a herd of 24 calves, after a single spraying applied with a Spray-Dip machine. This same formulation was found just as effective on 6 calves infested with the blood sucking louse, Linognathus vituli. It was used on one laboratory cow heavily infested with Hematopinus eurysternus, and no lice were found for a period of 40 days after treatment. At this time the results were somewhat complicated by the climatological conditions which would have brought about a natural reduction in the louse population, so no statement regarding eradication is being made. One-tenth percent aqueous spray of CO-RAL was also very effective in treating 3 swine infested with the hog louse, Hematopinus adventicius. No lice were found on these animals over a period of 65 days, which is considered to be virtually eradication.

Malathion emulsifiable concentrate spray of 0.5 percent appears to have eradicated a louse population on a "carrier" animal, when treated in the spring. The date of treatment was March 22, 1961. A replication of this test is needed in the fall or early winter to determine if eradication can actually be achieved. Treatments for lice applied in the spring are difficult to assess because of the natural population reduction that accompanies the advent of warm weather.

A herd of range Herefords, known to have been heavily infested with lice, Hematopinum eurysternus, in the winter, were sprayed in June 1960, with 0.375 percent Ruelene prepared from an emulsified concentrate. Lice were found 14 days after treatment on certain indicator animals, so the herd was re-sprayed, using 0.5 percent toxaphene emulsified concentrate spray. The cattle were examined about 6 months later (December 13, 1960) and no lice were found. They were again examined on January 18, 1961, and a total of 5 lice were found on 2 bulls. This was interpreted to mean that excellent control, but not eradication can be achieved by two sprayings, 14 days apart, using Ruelene and toxaphene sprays prepared from emulsified concentrates, if applied in the summer when the louse population is reduced by natural conditions, such as warm weather and the resulting loss of winter hair coat.

Field tests for the elimination of louse infestations in 2,000 head of cattle in Virginia were continued this fiscal year. This herd was originally infested with 4 species of lice in the spring of 1957, when the tests were started. At this time, the cattle were sprayed twice with lindane, applied with a Spray-Dip machine. In 1958, the treatment was modified by substituting malathion for lindane. The application of these treatments apparently eliminated Damalinia bovis and Solenopotes capillatus, but not Hematopinus eurysternus nor Linognathus vituli. In 1959 and 1960, the treatment regimen was again modified by using rotenone suspensions followed in 2 weeks by malathion. During the examination of the cattle in April 1961, L. vituli was present, as it has been through the tests; H. eurysternus absent, D. bovis absent, and S. capillatus present but in small numbers.

In 1962, at the Albuquerque laboratory, low level feeding of ronnel to cattle and sheep infested with psoroptic mites, and cattle infested with biting and blood-sucking lice, appeared to satisfactorily reduce louse populations when administered at the rate of 20 mg/kg/day for 34 days. Its effect on psoroptic mite infestations was less satisfactory. The indications are that this method of parasite control is less effective and economical than dipping or spraying.

Successful control of cattle lice in a herd of 2,500 cattle in Russell County, Virginia, was obtained with 0.25 percent CO-RAL WP spray, applied with a Spray-Dip machine. From the standpoint of biological efficiency and cost it was determined that one spraying with CO-RAL was not superior to two treatments with malathion applied at intervals of 10-12 days.

F. Methods for Control and Eradication of Ticks.

In 1961 and 1962 at the Albuquerque, New Mexico, station, preliminary dipping tests with insecticides for the control of ticks (Boophilus annulatus and B. microplus) infesting cattle in Mexico showed that satisfactory control could be obtained with a single treatment of CO-RAL, at concentrations of 0.25, 0.12, and 0.06 percent. Even at 14 days after dipping, 100 percent mortality was evident in cattle dipped in 0.25 percent concentration. Korlan was not as effective. Seven days after dipping only 50 percent mortality was obtained with 0.5 percent concentration. The animals were re-dipped and effective control was obtained. Delnav virtually eradicated infestations in 7 days, thus making it one of the most effective compounds used.

G. Control and Eradication of Scabies.

In 1961 at the Albuquerque, New Mexico, laboratory, small scale dipping tests for the control of psoroptic scab in sheep with several compounds classified as acaricides or acaricide-insecticides, were conducted at the Animal Disease and Parasite Research Laboratory at Albuquerque, New Mexico. To prove the effectiveness of the compounds, the treated animals were held for examination and observation for long periods, lasting in some instances for a year. The conclusions drawn from these tests were that none of the compounds, with the possible exception of 0.5 percent CO-RAL, was as, or more effective than Lindane, taken as a standard. The compounds tested were malathion, Tedion, Korlan, CO-RAL, Dylox, Bayer 22,408, Bayer 28,589, Bayer 30,686, Bercotox, Orthorix, Triethanolomine, and Sevin. Sheep were used in all but 2 tests. Sevin was used on a calf heavily infested with psoroptic mites, and 0.5 percent CO-RAL was used on swine infested with sarcoptic mites.

Attempts to transfer psoroptic mites infesting ears of goats to the ears and bodies of sheep failed insofar as it was not possible to produce infestations on sheep that would produce sequential generations of mites creating permanent infestations. Transfer of mites from goats to sheep were made manually, and attempts to transfer them naturally were made by keeping infested goats and clean sheep penned together. Some of the penned animals were kept together for more than a year without producing psoroptic scabies in the sheep.

Eighteen head of cattle heavily infested with psoroptic mites were sprayed with CO-RAL, using a Spray-Dip machine. Three animals were used as untreated controls. The aqueous suspension sprays used had concentrations of 0.125, 0.25, and 0.5 percent. After treatment the animals were isolated for observation and examination in groups based upon the spray concentrations used. At this date it is too early to assess the results. The animals will be kept under these conditions for additional examinations and observation throughout the coming summer, fall and winter.

Detailed examination of 11 sheep in June, when the winter-time lesions were completely resolved, was negative for the presence of live mites. Although such hiding places as inguinal fossae, ears, infra-orbital glands and inter-digital spaces were searched for mites, none were found. Despite the negative results, it is believed that a few individual sheep, going through the latent phase of scabies infestation, harbor sufficient mites over the summer months to form a nucleus of infection for the flock during the fall and winter when optimum conditions for the development prevail.

In Illinois, 8 toxaphene tests were started for the control of psoroptic sheep scab in 1959. These tests were carried through the fall, winter, and spring seasons of 1959-60. Observations on the treated flocks covered periods of from 3 to 18 months, and show that toxaphene dips, 0/5 percent concentration of active ingredient, applied once were highly effective in the control of psoroptic sheep scabies. Following these tests, 395 flocks, totalling 22,053 head, were treated by dipping in 0.5 percent toxaphene by State and Federal Livestock Inspectors in cooperation with the Animal Disease

and Parasite Research Division. The results showed that of the 395 flocks 11, or approximately 3 percent, subsequently showed clinical evidence of scabies, and required re-treatment. Live mites were found on 5 of the 11 flocks. Failure to control scabies in these flocks could not always be attributed to the treatment because reinfestation from untreated sheep was a possibility that could not be eliminated. The conclusions drawn were that 0.5 percent toxaphene, applied as dips to sheep infested with psoroptic mites under a variety of conditions, was a safe and effective method of controlling psoroptic sheep scab.

Ethion, an organic thiophosphate developed as a miticide, was found to be ineffective against psoroptic mites infesting sheep when used as dips having 0.1, 0.5, and 0.75 percent concentration of the active ingredient. Furthermore, the compound was found toxic to lambs and goats, but not to ewes at concentration levels of 0.75 and 0.5 percent.

In 1962, at the Albuquerque, New Mexico, laboratory, dipping tests with candidate compounds for sheep scabies (Psoroptes ovis) indicate that Malathion 0.5%, CO-RAL 0.25%, Diazinon 0.05%, Shell 4294 0.2%, and possibly Delnav, show the most promise as effective miticides. Additional tests using larger groups of animals are needed. CO-RAL at 0.25% spray for cattle scabies (Psoroptes bovis) shows promise as a means of controlling this parasite.

Tests to provide information on the protective period of lindane and toxaphene against infestation with P. ovis indicate these two compounds can provide protection under maximum mite population pressure for approximately 72 days.

Tests to determine the rate of depletion of gamma isomer in sheep dipping vats appear to have raised more questions than answers. Data collected indicate that depletion in dipping vats is probably due to selective removal by the wool of sheep rather than aging or contamination of vat samples.

A survey of the incidence of Chorioptea ovis in Southwestern sheep shows this parasite to be far more prevalent than usually believed. The significance of this finding is hard to interpret because in only 1 animal were mites associated with lesions.

Forty head of sheep going through the latent phase of psoroptic scabies were examined carefully for mites. Although the body areas known to have had active lesions during the winter and early spring were carefully examined, no mites were found on these areas. The likely body hiding places, such as the ears, nares, infraorbital, inguinal and interdigital spaces were carefully searched for mites but none were found. Later, with the advent of cooler weather, scabies infestation on these animals became apparent, and 6 weeks after the first symptoms were noted, the flock was 100 percent infested. This is taken to mean that latent scabies infestation in sheep can go undetected during the hot summer months in New Mexico. The nature of the infestation's survival is not presently very well understood.

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AREA NO. 15 - MISCELLANEOUS PARASITES AND PARASITIC DISEASES

Problem. Parasitism is a way of life that characterizes the majority of living things. Except for basic life processes, it is probably the commonest biological phenomenon. More than 50,000 kinds of animal parasites (i.e., parasites classified as animals as opposed to those classified as plants) are known. New varieties are being discovered and described at a rate of about 500 per year. Some devastating parasites, indigenous to foreign countries, threaten to surmount barriers imposed against them. Certain of these have already gained new footholds in livestock, poultry, and wildlife. Essential elements of procedure against parasites--established, exotic, or new--are accurate diagnosis, development of full knowledge about them, and research on effective control measures. The primary requirement is development through research of up-to-date knowledge of classification and identification supported by a complete reference collection of parasites, including type specimens and familiarity with global research already done. Basic investigations of parasitisms as biological phenomena are involved, especially in host-parasite relations, immunology, serology, ultrastructure, and other aspects of diagnosis and control. The problem is to develop and maintain up-to-date methods of identification and the essential, supporting reference collections, as well as complete parasitological information extracted from the world's scientific literature; investigate important phenomena and host-parasite systems not covered in specific host categories; and provide bases for detection and control that are adequate to meet existing and anticipated needs, through research on problems involving various parasites and hosts, including wild animals and birds important to agriculture.

USDA PROGRAM

The Department has a continuing long-term program for parasitologists, biochemists, and microbiologists, engaged in basic and applied research in this area. Research is being conducted on the following problems at the designated locations.

The Federal scientific effort devoted to research in this area totals 11 professional man years. This effort is divided among subheadings as follows:

Classification of parasites 2.0 at the Beltsville Parasitological Laboratory.

Maintenance of parasite collection 1.0 at the Beltsville Parasitological Laboratory.

Maintenance and publication of author, subject, and host index-catalogues 2.5 at the Beltsville Parasitological Laboratory.

Incidence of livestock parasites in the Southeast 0.5 at Tifton, Georgia.

Immunologic and other biologic approaches to the prevention and control of parasitic diseases 5.0 at the Beltsville Parasitological Laboratory.

RELATED PROGRAMS OF STATE EXPERIMENT STATIONS AND INDUSTRY

Work with miscellaneous parasites and parasitic diseases has no counterpart in the States. Other than in USDA, no complete parasite collections or indices of parasitological information are being developed and maintained in the United States. The USDA is almost the sole source of comprehensive information on identification, distribution, and economic importance of parasites and up-to-date treatment. Such information is furnished to workers in industrial concerns, experiment stations, colleges and universities, Federal and private research laboratories, and the Armed Forces.

REPORT OF PROGRESS FOR USDA AND COOPERATIVE PROGRAMS

A. Classification of parasites.

In 1961 at the Beltsville Parasitological Laboratory, two hundred and ninety-four lots of specimens were received for identification (95 nematodes, 31 cestodes, 22 trematodes, 42 arthropods, and 4 lots of miscellaneous material). Of particular interest was the identification of Rhipicephalus evertsi evertsi, the red tick, from an African antelope (eland) exhibited in an animal compound known as "Africa U.S.A." at Boca Raton, Florida. On further examination other animals in this zoo-like compound were found to be harboring the red tick. The red tick can transmit Theileria parva (the cause of East Coast fever), T. mutans, Babesia bigemina, B. equi, B. caballi, Rickettsia ruminantium (the cause of heart-water), R. conorii, and Borellia theileri. Three new helminths and a protozoan parasite were described in 6 papers published during the year.

In 1962, two hundred and seventy-seven lots of specimens were identified during the fiscal year (36 cestodes, 137 nematodes, 27 trematodes, 73 arthropods, 3 protozoans, and 1 lot of miscellaneous specimens). Among the cestodes were 3 Sparganum, an immature stage of the genus Spirometra, from the muscles of swine in Florida. This parasite is of human medical importance. Although reported in swine from other parts of the world, it has not been previously reported from swine in the United States. Observed inside one of the Sparganum was an Agamodistomum, an immature stage of a trematode, that also occurs in the muscles of swine.

Dracunculus insignis, a species very similar to one occurring in man in oriental and middle eastern countries has been received from dogs in Virginia and Florida, from a skunk in Maine, and from raccoons in Maryland. Specimens from the opossum, tentatively identified as D. fuelleborni, were received from Aberdeen, Maryland. Previous reports of this nematode in America are comparatively rare.

The tropical horse tick, Dermacentor nitens, has become established in Florida, having been found in several counties. Recently we received ticks for identification taken from a sick horse. The illness was diagnosed as piroplasmosis. The ticks removed from the sick animal were determined as Dermacentor nitens, the tropical horse tick. Previous to 1960 this tick was not known to occur in Florida.

B. Maintenance of parasite collection.

In 1961, at the Beltsville Parasitological Laboratory, five hundred and forty-five lots of specimens were catalogued in the Parasite Collection of the Laboratory - 384 were recorded in the Animal Parasite Collection and 161 in the U. S. National Museum Helminthological Collection.

Four hundred and forty-five inquiries and requests for information were handled during the period of this report. This covered the following subjects: Identification of specimens - 155; loan of specimens - 76; cataloguing of specimens - 53; manuscripts - 100, and miscellaneous - 61.

Four parasitologists from universities in the United States and Canada stayed from 1 to 8 weeks at the Laboratory to study specimens.

Approximately 17,000 specimen jars were checked for loss of preserving fluid and corrosion of covers. Fluid was added to, or covers replaced, on 75 percent of the specimen jars.

In 1962 a total of 1,750 lots of specimens composed of 117 cestodes, 574 nematodes, 883 trematodes, 164 arthropods, 5 protozoans, and 7 miscellaneous organisms, were assigned catalogue numbers, location numbers, and recorded in the parasite and host indexes of the Collection. This makes them immediately available for study and comparison with new specimens received for identification. Approximately two-thirds of the specimens recorded represent part of a large backlog of parasites that were received during preceding years for deposit in the Collection. Among the old specimens were parasites collected around 1890 from Australian domestic and wild animals, marine fish, American wildlife, and ticks used in experimental transmission of anaplasmosis.

In connection with maintaining the Collection, approximately 440 inquiries were answered during the year. The inquiries covered the following subject matter: Identification of specimens 138; loan of specimens 67; cataloguing of specimens 47; nomenclature 14; manuscripts 76, and miscellaneous 98.

During August and September 1961, the Collection was moved from the basement of Bldg. 318 to the basement of Bldg. 120, a distance of about 3 miles. This was accomplished without breakage or loss of specimens.

During the year numerous requests for identification of nematodes, acanthocephalans and linguatulids have been received from 22 States, including Alaska, Hawaii and the Canal Zone, as well as from Africa, Pakistan, Formosa, South America, Egypt and the Azores.

C. Maintenance and publication of author, subject, and host index-catalogs.

In 1961, at the Beltsville Parasitological Laboratory, the Index-Catalogue of Medical and Veterinary Zoology was maintained and expanded in its various sections: Author, Subject, and Host Catalogues, and Checklist of Specific and Subspecific Names. New entries augmenting the Catalogues were as follows: Author entries, 9,958; Parasite entries, 21,224; and Host entries, 7,557. The Index-Catalogue has continued to supply references for the Treatment Catalogue of the Antiparasitic Investigations Research Group and to index literature on plant parasitic nematodes. Added to the Host Catalogue have been 343 genera, 1,213 species and 421 subspecies previously unrecorded as hosts. This growth reflects in particular increased material on parasites of fishes and insects.

New genera and species of parasites were as follows:

	New genera	New species
Protozoa	20	146
Trematoda	45	293
Cestoda	8	76
Nematoda	49	279
Arthropoda and misc.	110	397
Total	232	1,201

The Index-Catalogue has had approximately 103 visits by specialists of the United States, and at least 11 other countries, using it as a source of information. Two parasitologists from Japan were consulting it for the entire year.

In 1962, new entries augmenting the Catalogues were as follows: Author entries, 7,782, Parasite entries, 13,598, and Host entries, 4,240. The Index-Catalogue has continued to supply references for the Treatment Catalogue of the Antiparasitic Investigations Section and to index literature on plant parasitic nematodes.

New genera and species of parasites are as follows:

	New genera	New species
Protozoa	7	203
Trematoda	43	331
Cestoda	12	79
Nematoda	22	276
Arthropoda and misc.	23	411
Total	107	1,300

The Index-Catalogue has had approximately 120 visits by specialists of the United States and at least 15 other countries, using it as a source of information. Two parasitologists from Japan visited the Index-Catalogue from July 1, 1961, to May 25, 1962, and used it as their source for material for publication of monographs on parasites.

D. Incidence of livestock parasites in the Southeast.

In 1961, at the ADP Division Laboratory at Tifton, Georgia, 43 postmortem examinations were made of pigs and two tissue samples were examined for parasites. Twenty of these cases had parasite worm counts high enough to consider parasitism as the prime factor causing unthriftiness, scouring, etc. Twelve of these involved predominantly Strongyloides ransomi, three Ascaris suum, three of both of these species, and one involving these, plus Oesophagostomum sp. One of the tissue sections (small intestine) was heavily parasitized with Strongyloides ransomi and the other tissues examined contained Physocephalus sp.

For the third year the single most important parasite in pigs was Strongyloides ransomi. This has become particularly important in baby pigs which are becoming infected prior to birth. Adult worms have been recovered from the small intestines of baby pigs only 2 days old. These pigs were scouring from the time of birth. One 4-month-old pig harbored 1,288 Ascaris suum, all of which were young, immature forms. These were recovered from the oesophagus, stomach, small and large intestines, liver and gall bladder. Thirty fecal samples were examined. Sixteen were positive and 14 were negative. Strongyloides ransomi eggs were present in moderate to high counts in all 16 cases, Ascaris suum in one case, and Oesophagostomum sp. in three cases.

Twenty-nine cattle fecal samples were examined during the year for evidence of parasitism. Twenty-one were positive and eight were negative. Of the positive cases, only three had high enough egg counts to be considered of possible clinical significance. These were in three young dairy calves which were passing eggs of Strongyloides papillosus. Fourteen postmortem cases were examined for parasites and all but one were positive. Eight of these had severe damage to the stomachs and the predominant causative agent in seven was determined to be Ostertagia ostertagi. The eighth case showed the general appearance of "bottle jaw" and the stomach was extremely edematous and had many necrotic ulcers that appeared healed. Approximately 300 O. ostertagi were recovered, but no Haemonchus placei which usually causes this condition.

Two cases of particular interest were from a 5-year-old, and a 9-year-old brood cow which had severe infections of O. ostertagi. The two cows were from adjacent farms and were brought to the laboratory on the same day. The stomachs of both were slightly edematous and the mucosal surface was covered entirely with small fibrous nodules and tiny petechial and minute haemorrhagic ulcers. The 5-year-old cow had 1,120,000 O. ostertagi and the 9-year-old cow had 878,000 O. ostertagi.

Haemonchus placei was recovered from three clinical cases, Cooperia punctata from four, and Trichostrongylus axei from two cases.

Twenty-seven fecal samples from horses were examined. Twenty-four were positive for strongyle larvae, but all in low numbers.

Twenty-eight fecal samples from dogs were examined. Twenty-three were positive and the eggs indicated hookworm as the predominant parasite present.

This project was discontinued November 3, 1961.

E. Immunologic and other biologic approaches to the prevention and control of parasitic diseases.

In 1961 at the Beltsville Parasitological Laboratory, studies were continued on the cultivation of Oesophagostomum radiatum in a medium containing principally trypticase, yeast extract, glucose, cattle serum and extracts of rabbit embryo and pig liver, and in modifications formulated by the addition or deletion of ingredients. Cultures were examined at 1- to 4-day intervals during an incubation period of 24 days at 38.5 C.

Preliminary results on the rate of development in 8 variations indicated that all ingredients of the original medium are essential except swine liver extract. Indeed, larvae grown in media without this constituent equaled or surpassed the rate of development of those grown in its presence. Larvae cultured in the absence of serum showed little or no growth after 24 days incubation. When certain substitutes were added in place of serum, growth and development occurred but was delayed.

Hyostrongylus rubidus, Oesophagostomum quadrispinulatum, Oes. radiatum, Hamonchus contortus (bovine), Ostertagia ostertagi, and Cooperia spp. were cultured to fourth stage in a medium containing principally trypticase, yeast extract, glucose, sheep or cattle serum, and extracts of rabbit embryo and pig liver. Two ovine strains of H. contortus, one resistant and the other non-resistant to phenothiazine, reached fourth molt in the same medium. Addition of a vitamin supplement to some of the cultures accelerated development, but in vitro growth of all species was slower than that in vivo.

The influence of tissue extracts and sera from various sources on the growth of Oesophagostomum radiatum was studied by adding these materials to a medium comprised largely of trypticase, yeast extract, glucose, and extracts of rabbit embryo and pig liver. Extracts of the small intestine of a helminth-free calf (C-1) and serum from a helminth-free yearling (C-2) had little effect on development to early fourth stage. A distilled water extract of the intestinal mucus and ingesta from C-2 and from C-3 (yearling patent with Oes. radiatum) accelerated growth markedly while that from C-4 (yearling resistant to Oes. radiatum) had a lesser effect. A definite lag in development occurred in media containing sera and extracts of the cecum and colon from C-3 and C-4. In all cultures containing tissue extracts of C-3 and C-4, most larvae had precipitates at the body openings and were covered with a coating of media debris.

Studies were continued on the evaluation of the agar diffusion precipitin test as a method of diagnosing prepatent kidney worm infections in swine. Sera obtained from swine in a program for management control at Tifton, Georgia, were tested as were sera obtained from kidney worm-free herds at Beltsville, and from swine infected experimentally. Results indicate that the method is reliable, even for low-level infections, when tests are made on a number of consecutive serum samples.

Analytical studies revealed little evidence of urea or uric acid in extracts of the excretory glands of the swine kidney worm (Stephanurus dentatus). They showed, however, the presence of proteins, some of which proved to be antigenic in the agar diffusion precipitin test for the diagnosis of kidney worm infections. Compared to other parts of the worm body, the excretory gland contained a high proportion of the soluble proteins and these showed most of the precipitinogenic activity. In addition to the excretory glands, the esophagus, head, and intestine gave strong precipitin lines. The body fluid gave very weak lines, and the cuticle and gonads were negative.

In limited trials, the number of Oesophagostomum radiatum, Ostertagia ostertagi, and Cooperia oncophora, developing to adult males in susceptible hosts, was inversely proportional to x-ray doses ranging from 10,000 to 22,800 roentgens administered to the infective larvae. Moreover, the total number of Ostertagia and Cooperia that became established was significantly reduced as compared to a control which received non-irradiated larvae. Although egg counts were reduced and some abnormal ova were observed, the F_1 generation of O. radiatum produced an apparently "normal" infection in a susceptible host.

In 1962, at the Beltsville Parasitological Laboratory, phosphate buffer extracts of organs and tissues of Stephanurus dentatus, which had previously been shown to contain specific precipitinogens, were injected into swine in an attempt to produce immunity to subsequent challenge. One, 2, or 3 injections, spaced 1 week apart, were followed by challenge with 1,000 infective kdiney worm larvae given 7, 14, 21, or 28 days after the last injection. Principals and controls challenged at the same time were necropsied 28 to 30 days later, and the degree of infection graded by the number and gross appearance of the liver lesions.

Partial protection was demonstrated when single injections of either excretory gland extract or intestinal tissue extract or a combination of both were followed by challenge after 14 days. Swine challenged before or after this period were comparable to controls in most cases and occasionally showed greater pathology. Multiple injections did not provide a greater degree of protection than single injections regardless of the time interval before challenge.

Stephanurus dentatus developed to fourth stage in 15 days in a complex medium (SM-1) containing principally trypticase, yeast extract, glucose, extracts of rabbit embryo and pig liver, and 10 percent cattle serum. Identical development was also achieved in 3 modifications formulated by the addition of: double amounts of swine liver extract and bovine serum; a double amount of swine liver extract; and a double amount of autoclaved swine liver extract. In Pitts' medium, which differs principally from SM-1 and its modifications by an increased amount of bovine serum (25 percent) and the absence of tissue extracts, developmental time to fourth stage was reduced.

Studies on the effect of ionizing radiation on the infective larvae of Oesophagostomum radiatum were continued. As previously reported a selective lethal action on males was demonstrated which could be correlated with the total dosage. The presence of vaginal plugs as an evidence of fertilization of female worms was adopted as a criterion for estimating male development after irradiation. At a dose of 9,000 r most males reached maturity, mated, and died before necropsy of the host. However, at a dose of 13,000 r most males died before reaching maturity.

Cooperia punctata was cultured from artificially exsheathed third-stage larvae to immature adults in a medium containing principally trypticase, yeast extract, glucose, bovine serum, and extracts of rabbit embryo and pig liver. Similar development was obtained in modifications formulated by the addition of intestinal tissue extracts from bovines parasitized with Oesophagostomum radiatum or extracts of rumen contents, ingesta and mucus, or intestinal tissue from a calf parasitized with C. punctata. Fifth-stage was reached in all media in 13 to 19 days but survival, particularly after reaching fourth-stage, was poor.

Antibodies were demonstrated in the serum of a calf infected with Cooperia punctata by using living infective third-stage larvae and in vitro grown parasitic larvae of this nematode as sources of antigen. The visible reactions, larval precipitates and cuticular coating, which were strong during patency and weak after patency had ended, became very strong 4 days after challenge with C. punctata and remained strong up to 50 days after challenge.

Using "immune" serum in an agar double diffusion technique, precipitinogens were demonstrated in extracts of adult Oesophagostomum radiatum collected from bovines but not in extracts of larvae grown in vitro. Stronger reactions were obtained with the serum from a multiply infected calf than with serum from a singly infected calf.

Ultracentrifugation through sucrose gradients of swine serums, showed the absence of serologically-active macroglobulins in serums from swine infected with kidney worms as well as from noninfected swine; antibodies to kidney worm antigens in extracts of kidney worm excretory glands sedimented slowly while specific antigens sedimented more rapidly, indicating the possibility of separating nonspecific and specific antigens by this method. The main components of extracts of kidney worm excretory glands and intestines were found to have sedimentation coefficients of 3.6 S and 13.5 S, and 2.8 S and 12.2 S, respectively.

Absorption studies failed to prove conclusively the occurrence of common antibodies to kidney worm antigens in serums from infected swine and in serums from noninfected swine.

Electrophoresis in agar indicated that nonspecific and specific antigens of the swine kidney worm can be separated.

Under PL 480 projects, work was reported as follows:

Control of the liver fluke, Fasciola hepatica in domestic ruminants. Pulawy, Poland.

Hexachlorophene was not considered to be satisfactory for mass field use for anti-fluke therapy largely because of its toxic properties. The intramuscular inoculation of carbon tetrachloride, although of high therapeutic value, was unsuitable because of the severe reaction at the point of inoculation.

Studies on the giant liver fluke, Fasciola gigantica. Veterinary Faculty, Ankara University, Ankara, Turkey.

Fasciola gigantica and its intermediate snail host, Lymnaea auricularia, were collected in the southern and western parts of Turkey. The snail was also found in the eastern provinces but fascioliasis was not found by examining livers at slaughter houses. If infected ruminants were to be introduced to pastures in this region, this part of the country might also become a problem area. Hexachlorophene destroyed all flukes in sheep and goats when administered 30 to 119 days after experimental exposure. Treatments with carbon tetrachloride did not give consistently good results. Six sheep, exposed to about 100 Fasciola gigantica metacercariae, died between the 87th and 113th days after exposure. At necropsy flukes were found in the livers and the livers had hematomas. The prepatent period of F. gigantica was 102 to 115 days in sheep, 87 days in goats, and 132 days in one buffalo calf.

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Line Project Check List -- Reporting Period January 1, 1961 to June 30, 1962

Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP al	Investigation of Infectious and Non-infectious Diseases of Cattle			
ADP al-3(R)	Investigations of Brucellosis in Cattle	Beltsville, Md.	yes	1-A
		Ames, Iowa	yes	1-A
		St. Paul, Minn.	yes	1-A
		Madison, Wisc.	yes	1-A
		College Park, Md.	yes	1-A
ADP al-4(R)	Investigations of Paratuberculosis (Johne's Disease) of Cattle	Auburn, Alabama	yes	1-B
		Ames, Iowa	yes	1-B
ADP al-9(R)	Investigations of Vibriosis of Cattle	Beltsville, Md.	yes	1-C
		Ames, Iowa	yes	1-C
		Ithaca, New York	yes	1-C
ADP al-12	Investigations of Anaplasmosis of Cattle	Beltsville, Md.	yes	10-N
		Kerrville, Texas	yes	10-N
ADP al-13(R)	Investigations of Tuberculosis of Cattle	Auburn, Alabama	yes	1-D
		Ames, Iowa	yes	1-D
ADP al-14(R)	Mucosal-Respiratory Disease-Complex of Cattle	Lafayette, Ind.	yes	1-E
		Davis, Calif.	yes	1-E
		Fort Collins, Colo.	yes	1-E
		Ames, Iowa	yes	1-E
ADP al-15	Investigations of Mastitis of Cattle	Beltsville, Md.	yes	1-F
		Ames, Iowa	yes	1-F
		Davis, Calif.	yes	1-F
ADP al-17	Investigations of Respiratory Diseases of Cattle (Shipping Fever)	Beltsville, Md.	yes	1-G
		Ames, Iowa	yes	1-G
ADP al-18	Investigations of Leptospirosis of Cattle	Beltsville, Md.	yes	1-H
		Ames, Iowa	yes	1-H
ADP al-19	Investigations of Infectious Causes of Infertility in Cattle	Beltsville, Md.	no	
		Ames, Iowa	no	
ADP al-20	Investigations of the survival and inactivation of foot-and-mouth disease virus in meat and meat by-products	Greenport, L. I. New York	yes	8-R
ADP al-21	Epizootic Bovine Abortion	Davis, Calif.	yes	1-I
ADP al-22*	Foot Rot (Infectious Pododermatitis) of Cattle	Ames, Iowa	no	
ADP al-23	Cytological investigations. Studies on the biological mechanism of natural resistance and susceptibility to foot-and-mouth disease virus	Greenport, L. I. New York	yes	8-S

* Initiated during the reporting period

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP a2	Investigations of Infectious and Non-infectious Diseases of Swine.			
ADP a2-3(R)	Investigations to determine the cause of cholera outbreaks in swine immunized against hog cholera .	Ames, Iowa	yes	2-A
ADP a2-8(R)	Studies on the causative agent (or agents), mode of spread, diagnosis and control of atrophic rhinitis in swine.	Beltsville, Md. Ames, Iowa	yes yes	2-B 2-B
ADP a2-10(R)	Investigation of transmissible gastro-enteritis (TGE) complex of young pigs.	Ames, Iowa Davis, Calif. Lafayette, Ind.	yes yes yes	2-C 2-C 2-C
ADP a2-11	Studies of hog cholera virus propagated in vitro.	Ames, Iowa	yes	2-A
ADP a2-12	Investigation of factors influencing the potency and immunizing ability of hog cholera vaccines in individual pigs and in groups of pigs.	Ames, Iowa	yes	2-A
ADP a2-13	Pilot field studies to evaluate modified live-virus hog cholera vaccines.	Ames, Iowa Live Oak, Fla. Valdosta, Ga.	yes yes yes	2-A 2-A 2-A
ADP a2-15	Investigations of erysipelas of swine.	Beltsville, Md. Jersey City, N. J.	yes yes	2-D 2-D
ADP a2-16	Investigations of brucellosis of swine.	St. Paul, Minn.	yes	2-E

Line Project Check List -- Reporting Period January 1, 1961 to June 30, 1962

Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP a3	Infectious and Non-Infectious Diseases of Sheep and Goats			
ADP a3-1(R)	Investigations of vibriosis of sheep	Fort Collins, Colo. Bozeman, Montana Logan, Utah	yes yes yes	3-C 3-C 3-C
ADP a3-3	Investigations of scrapie of sheep	Compton, England Edinburgh, Scotland	yes yes	3-B 3-B
ADP a3-4	Viral ulcerative dermatosis of sheep	Fort Collins, Colo.	yes	3-D
ADP a3-5	Investigations of bluetongue in sheep-diagnosis, transmission and control	Denver, Colorado Pullman, Washington	yes yes	3-A 3-A

Line Project Check List -- Reporting Period January 1, 1961 to June 30, 1962

Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP a4	*Investigations of Infectious and Non-Infectious Diseases of Horses			
	*No research work is being done with horses. No line projects were in effect during this reporting period, and none are in effect as of this date.			

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP a5	Investigations of Infectious and Non-infectious Diseases of Poultry			
ADP a5-2(R)	Investigations of Salmonellosis of Poultry	Ames, Iowa	no	5-B
ADP a5-16	Investigations of Pasteurellosis of Poultry	Ames, Iowa	yes	5-C
ADP a5-17	Investigations of chronic respiratory disease complex in chickens and turkeys	Storrs, Conn.	yes	5-D
		Newark, Del.	yes	5-D
		Athens, Ga.	yes	5-D
		College Park, Md.	yes	5-D
		Amherst, Mass.	yes	5-D
		Ithaca, New York	yes	5-D
		Raleigh, N. C.	yes	5-D
		College Station, Texas	yes	5-D
		Blacksburg, Va.	yes	5-D
		St. Paul, Minn.	yes	5-D
		Hebrew Univ., Israel	yes	5-D
ADP a5-18	Investigations of Newcastle Disease	Athens, Ga.	yes	5-E
		Ames, Iowa	yes	5-E
		Orono, Maine	yes	5-E
		Madison, Wisc.	yes	5-E
		Pulawy, Poland	yes	5-E
ADP a5-19(C)	Bluecomb in Turkeys	St. Paul, Minn.	yes	5-F
ADP a5-20	Ornithosis in Poultry	Davis, Calif.	yes	5-A
		St. Paul, Minn.	yes	5-A
		Corvallis, Ore.	yes	5-A
		College Station, Texas	yes	5-A
		Ames, Iowa	no	
ADP a5-21	Turkey Airsacculitis	Ames, Iowa	yes	5-D
		St. Paul, Minn.	yes	5-D
		Madison, Wisc.	yes	5-D
ADP a5-22*	Study of Avian Leukosis	Ithaca, New York	no	
- - - - -	- -			
ADP a10	Foot-and-Mouth and Other Exotic Diseases of Poultry			
ADP a10-1	Adaptation of the virus of foot-and-mouth disease to poultry and embryonating chicken eggs	Greenport, L. I. New York	yes	5-G

*Initiated during reporting period

Line Project Check List -- Reporting Period January 1, 1961 to June 30, 1962

Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP a6	Infectious and Non-infectious Diseases of Fur Animals, Including Rabbits			
ADP a6-2(R)*	Field and Laboratory Studies of Diseases of Fur Animals	Pullman, Washington	yes	6-A
ADP a6-5	Enteric disease-complex of Rabbits	Fontana, California	yes	6-B
ADP a6-6	Respiratory disease-complex of rabbits	Fontana, California	yes	6-C
ADP a6-7**	Field and Laboratory Studies of Diseases of Fur Animals	Pullman, Washington	yes	6-A
ADP a6-8**	Studies on the persistence and transmission of viral and rickettsial diseases in helminths associated with diseases of fur animals	Pullman, Washington	yes	6-D

* Superseded by ADP a6-7
** Initiated during reporting period

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP a7	Miscellaneous Infectious and Non-infectious Diseases of Animals			
ADP a7-5(R)	Reservoirs, transmission and immunological studies of vesicular stomatis	Ames, Iowa	no	
ADP a7-7(R)	Investigation of livestock poisoning by plants, their toxicity for different classes of livestock, and methods of treatment and prevention	Logan, Utah	yes	7-I
		Sao Paulo, Brazil	yes	7-I
ADP a7-8(R)	Investigation of the toxicity of herbicides and herbicide-treated plants to livestock	Logan, Utah	yes	7-J
ADP a7-11(R)*	Toxicological and pathological studies in livestock of insecticides	Kerrville, Texas	yes	7-E
ADP a7-12(R)	Use of radioactive isotopes in studying insecticide toxicology in animals	Kerrville, Texas	yes	7-E
ADP a7-13(C)**	Response of animals to daily intake of radioisotopes with particular reference to movement in the food chain and pathological response	Ithaca, New York	no	
ADP a7-14	Fractionation, purification and characterization of the components of normal and immune sera of animals	Ames, Iowa	yes	7-B
ADP a7-15***	Investigations of bloat in ruminants	Ames, Iowa	yes	7-C
		Davis, Calif.	yes	7-C
		College Park, Md.	yes	7-C
		St. Paul, Minn.	yes	7-C
		State College, Miss.	yes	7-C
		Ithaca, New York	yes	7-C
		Madison, Wis.	yes	7-C
ADP a7-16	Preparedness for Laboratory Assistance in Diagnosis of Foreign Animal Diseases	Greenport, L. I. New York	yes	7-D
ADP a7-17	Studies to develop alleviators and diagnostic tests for plant poisoning and methods to avoid harmful residues in animal tissues from ingesting chemically treated plants	Logan, Utah	yes	7-K
ADP a7-18****	Investigations in cattle and sheep of the biochemical effects of agricultural chemicals and control substances	Kerrville, Texas	yes	7-F
ADP a7-19****	Investigations of detoxication mechanisms in cattle and sheep	Kerrville, Texas	yes	7-G
ADP a7-20	Characterization of cytological responses to toxic actions of antiparasitic and other agricultural chemicals in cattle and sheep tissues	Kerrville, Texas	yes	7-H
ADP a7-21	Susceptibility of wild animals to foot- and moutn disease	Greenport, L. I. New York	yes	7-L

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Line Project Check List -- Reporting Period January 1, 1961 to June 30, 1962

Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP a7	Miscellaneous Infectious and Non-infectious Diseases of Animals, cont'd			
ADP a7-22****	Studies of the incidence and pathology of cancer and other tumors in food-producing animals	Denver, Colo. Ames, Iowa	yes yes	7-A 7-A
ADP a7-23****	Toxicological and pathological effects of insecticides, herbicides, fungicides, and other agricultural chemicals on livestock and poultr	Kerrville, Texas	yes	7-E

* * Superseded during reporting period by ADP a7-23
** Discontinued during reporting period
*** Cooperative agreement at Minnesota discontinued during reporting period
**** Initiated during the reporting period

Line Project Check List -- Reporting Period January 1, 1961 to June 30, 1962.

Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP a8	Foot-and-Mouth and Other Exotic Infectious Diseases of Cattle			
ADP a8-1	Pathological investigations of foot-and-mouth disease in cattle	Greenport, L. I. New York	yes	8-A
ADP a8-2	Application of fluorescent antibody technique to investigations of foot-and moutn disease in cattle	"	yes	8-B
ADP a8-3*	Diagnostic investigations of foot-and-mouth disease-Refinement of serological tests	"	yes	8-C
ADP a8-4*	Diagnostic investigations. Determine susceptibility of cell lines in tissue culture to the various types and varients of foot-and-mouth disease virus, vesicular stomatitis and vesicular exanthema	"	no	
ADP a8-5*	Diagnostic investigations of foot-and-mouth disease. Production and maintenance of standardized reference stocks of specific strains of virus and homologous antisera	"	no	
ADP a8-6	Immunological investigations of foot-and mouth disease. Study of the carrier state in convalescent animals	"	no	
ADP a8-7*	Immunological investigations of foot-and-mouth disease. Role of parasites in transmission of virus and the influence of parasitism on clinical and immunogenic responses of host animals	"	no	
ADP a8-8	Immunological investigations. Vaccine studies on foot-and-mouth disease	"	yes	8-D
ADP a8-9*	Immunological investigations. Study of antigenic variations of foot-and-mouth disease virus	"	yes	8-E
ADP a8-10	Immunological investigations of foot-and-mouth disease. Study of antibody production in vitro.	"	no	
ADP a8-11	Immunological investigations. Immune response to various types and sub-types of foot-and-mouth disease virus	"	yes	8-F
ADP a8-12	Quantity production of foot-and-mouth disease virus by tissue culture methods	"	yes	8-G
ADP a8-13	Cytological investigations. A micro-cinematographic study of the effects of foot-and-mouth disease virus on susceptible cells in tissue culture	"	yes	8-H
ADP a8-14	Cytological investigations of foot-and-mouth disease. The establishment of pure stable lines of cultured cells	"	yes	8-I
ADP a8-15	Isolation, identification, concentration and purification of foot-and-mouth disease virus	"	yes	8-J

Continued on next page

Line Project Check List -- Reporting Period January 1, 1961 to June 30, 1962

Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP a8	Foot-and-Mouth and Other Exotic Infectious Diseases of Cattle - continued			
ADP a8-16	Chemical and physical characteristics of purified foot-and-mouth disease virus	Greenport, L. I. New York	yes	8-K
ADP a8-17	Mechanism of the interaction between foot-and-mouth disease molecules and host cells	"	yes	8-L
ADP a8-18	Investigations of the genetic biochemistry of foot-and-mouth disease virus	"	yes	8-M
ADP a8-19	Effects of certain chemicals and physical environments on foot-and-mouth disease virus	"	yes	8-N
ADP a8-20	Microbiological investigations. Preservation of foot-and-mouth disease virus in suspension on freeze-dried materials with supporting additives and held at -50, -10, 4, 25, and 37°C.	"	yes	8-O
ADP a8-23	Investigations of Rinderpest of cattle	"	yes	8-P
ADP a8-24	Survival and transmission of foot-and-mouth disease virus in the semen of susceptible species of animals	"	yes	8-Q

*Discontinued during reporting period

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Work & Line Project Numbers	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP a9	Foot-and-Mouth and Other Exotic Diseases of Swine			
ADP a9-1	Immunological investigations of foot-and-mouth disease of swine	Greenport, L. I. New York	yes	9-A
ADP a9-2	Investigations of African Swine Fever (ASF)	Kenya, East Africa Madrid, Spain	yes yes	9-B 9-B

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP bl	Parasites and Parasitic Diseases of Cattle			
ADP bl-6(R)	Ecological factors influencing gastro-intestinal nematodes of cattle	Auburn, Alabama Experiment, Ga.	yes yes	10-A 10-A
ADP bl-7*	Effects of Mixed Helminth Infections	Auburn, Alabama	yes	10-B
ADP bl-9(R)	Acquisition of Parasites from Pastures	Beltsville, Md.	yes	10-C
ADP bl-12(R)	Effect of Pasture Mixtures and Pasture Management on Control of Internal Parasites	Auburn, Alabama Experiment, Ga.	yes yes	10-D 10-D
ADP bl-17(R)	Winter Coccidiosis (Bloody Scours) of Cattle	Logan, Utah Bozeman, Montana	yes yes	10-E 10-E
ADP bl-19(R)	Influence of Diet and Nutrition of Cattle on Roundworms	Beltsville, Md.	yes	10-F
ADP bl-20*	Interrelationship of Type of Pasture and Internal Parasites	State College, Mississippi	no	
ADP bl-22	Artificial Propagation of Protozoan Parasites	Beltsville, Md.	yes	10-G
ADP bl-23(R)	Host-Parasite Relationship of Coccidia	Auburn, Alabama	yes	10-H
ADP bl-24	Ecology and Immunology of the Cattle Lungworm	Beltsville, Md.	yes	10-I
ADP bl-25	Clinical and Physiological Aspects of Roundworm Parasitism in Cattle, including Anthelmintic Treatment	Davis, Calif.	yes	10-J
ADP bl-26**	Investigations of Trichomonad Parasites	Logan, Utah	yes	10-K
ADP bl-27**	Host-Parasite Relationships of Intestinal Worms, Cooperia, spp. in Cattle	Auburn, Alabama	yes	10-L
**	Investigations on Anaplasmosis, Piroplasmosis, babesiellosis of cattle	Montevideo, Uruguay	no	
**	Investigations on pathogenesis of lesions produced by the local leech, Limmatis nilotica	Jerusalem, Israel	no	

*Discontinued during reporting period
**Initiated during reporting period

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP b2	Parasites and Parasitic Diseases of Swine			
ADP b2-2(R)	Evaluation of the role of parasites in the economy of swine production	Beltsville, Md. Tifton, Georgia	yes yes	11-A 11-A
ADP b2-4(R)	The effect of anthelmintic treatment on rate of gain when administered to parasitized pigs of different ages and on different nutrition levels	Tifton, Georgia	yes	11-E
ADP b2-10(R)	Investigation of the bionomics and pathogenicity of the swine whipworm	Beltsville, Md.	yes	11-B
ADP b2-11(R)	Control of swine kidney worms by herd management under typical south Georgia farm conditions	Tifton, Georgia Raleigh, N. C.	yes yes	11-C 11-C
ADP b2-12(R)	Investigations of the swine intestinal roundworm, Ascaris suum	Lincoln, Nebr.	yes	11-F
ADP b2-15	Investigations of strains of Trichinella spiralis resistant to heat and cold and modes of transmission of the parasite	Beltsville, Md.	yes	11-D

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP b3	Parasites and Parasitic Diseases of Sheep and goats			
ADP b3-4(R)*	The life histories, biology, pathogenesis and control of certain helminth parasites of sheep occurring in the Southwest	University Park New Mexico	yes	12-F
ADP b3-7(R)	Effect of intestinal roundworms on the exogenous and endogenous utilization of protein, carbohydrate, and various minerals by sheep	Fargo, N. Dakota	yes	12-H
ADP b3-12**	Investigations of lungworms and lungworm disease of sheep and goats	Beltsville, Md.	yes	12-A
ADP b3-13	Investigations of parasitism of sheep in the South	Auburn, Alabama State College, Miss.	yes yes	12-E 12-E
ADP b3-14	Investigations of the bionomics of coccidial parasites of sheep and goats	Beltsville, Md.	yes	12-B
ADP b3-15	Investigations on the effects of helminthic infections on serum proteins of sheep and goats	Beltsville, Md.	yes	12-C
ADP b3-16	Investigations of gastrointestinal nematodes and nematodiases of sheep and goats and measures for their control	Beltsville, Md. Lexington, Ky.	yes yes	12-D 12-D
ADP b3-17	The biology of the liver fluke, Fasciola hepatica, of sheep and cattle with special reference to the free-living and intra-molluscan stages	Bozeman, Montana	yes	12-G
ADP b3-18***	The life histories, biology, pathogenesis and control of several helminth parasites of sheep occurring in the Southwest	University Park, New Mexico	yes	12-F
ADP b3-19***	Studies on the life cycles of Eimeria ahasta and Eimeria crandallis, pathogenic coccidia of sheep	Auburn, Alabama	yes	12-E

* Superseded by ADP b3-18
** Discontinued during reporting period
*** Initiated during reporting period

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP b4	Parasites and Parasitic Diseases of Poultry			
ADP b4-6	The Bionomics of Intestinal Protozoan Parasites of Poultry	Beltsville, Md.	yes	13-A
ADP b4-8	Investigations of the Immunology of Protozoan Parasitic Diseases of Poultry with Special Reference to Blackhead	Beltsville, Md.	yes	13-B
ADP b4-9	Investigations for Controlling Coccidiosis of Poultry	Beltsville, Md.	yes	13-C
ADP b4-10	The Biology of the Nematode Parasites of Poultry and related birds with Special Reference to the Application of Findings to Control Measures	Beltsville, Md.	yes	13-D

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP b5	Treatments for Removal or Control of Parasites of Domestic Animals			
ADP b5-5(R)	Evaluation, development, and standardization of chemical methods of established or reported value for the control of parasitic diseases of livestock and poultry	Beltsville, Md.	yes	14-A
ADP b5-6(R)	Develop new and improved anthelmintics for farm animals	Beltsville, Md. Auburn, Alabama	yes yes	14-B 14-B
ADP b5-7(R)	Investigations of chemical prevention of parasitism in livestock	Beltsville, Md.	no	
ADP b5-12(R)	Investigations of parasitic and related skin diseases of cattle, sheep, and swine, with primary emphasis on chemical control and basic biology of mange and scabies	Albuquerque, New Mexico	yes	14-D
ADP b5-13	Pathobiology of parasitic infections with special reference to the injuriousness of arthropod parasites, and the economic gain and efficiency of control measures	Albuquerque, New Mexico	yes	14-E
ADP b5-14	Development of new methods for the control and eradication of ticks of domestic animals, with special reference to the cattle fever ticks, Boophilus annulatus and B. microplus, the principal vectors of bovine piroplasmosis	Albuquerque, New Mexico	yes	14-F
ADP b5-15	Development of new approaches and methods for the control and eradication of scabies in sheep and cattle	Albuquerque, New Mexico	yes	14-G
ADP b5-16	Control of internal parasites of livestock by management practices that will not create consumer residue hazards	Auburn, Alabama	yes	14-C
ADP b5-17	Investigations of antiparasitic agents and measures for the control of parasites belonging to the family Oestridae	Albuquerque, New Mexico	yes	14-D

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Work & Line Project Number	Work and Line Project Titles	Work Locations During Reporting Period	Line Project Inc. in Summary of Progress	Line Project Inc. in Area & Subheading
ADP b6	Miscellaneous Parasites and Parasitic Diseases			
ADP b6-2(R)	Identification of parasites of importance in regulatory and other work	Beltsville, Md.	yes	15-A
ADP b6-3(R)	Taxonomic investigations of helminths and other parasites	Beltsville, Md.	yes	15-B
ADP b6-5(R)	Maintenance of author, subject, host, and anthelmintic catalogues and checklist of specific and subspecific names	Beltsville, Md.	yes	15-C
ADP b6-6(R)	Maintenance of Parasite Collections	Beltsville, Md.	yes	15-B
ADP b6-8*	Incidence of livestock parasites in the Southeast	Tifton, Georgia	yes	15-D
ADP b6-9	Publication of author, subject (parasite) and host index-catalogues of medical and veterinary zoology	Beltsville, Md.	yes	15-C
ADP b6-10	Investigation of immunologic and other biologic approaches to the prevention and control of parasitic diseases	Beltsville, Md.	yes	15-E
ADP b6-11**	Studies of the chemical and physical elements of parasites and parasite-host relationships in animals	Beltsville, Md.	no	
	Control of the liver fluke, Fasciola hepatica in domestic ruminants	Pulawy, Poland	yes	15-E
	Studies on the giant liver fluke, Fasciola gigantica	Ankara, Turkey	yes	15-E

* Discontinued during reporting period
** Initiated during reporting period